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CHEMISTRY DEPARTMENT

A Study of Some Animal and Vegetable Fats

Thesis

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by

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Summary of Ph.D. Thesis

A Study of Some Animal and Vegetable Fats - William C. Russell.

Part I - Analysis of Animal Fats

Many investigators in this field have compared the relative abundance of vegetable fat analyses with the scant information available on animal fats and have stressed the need for further analyses of the fats from a wider variety of animals. This study is concerned with the analyses of eight animal fats viz. those from crocodile, ostrich, flamingo, rabbit, mouse, porcupine, antelope and sea lion. The results obtained have been compared with those derived from the fats of related animals, showing that in general, the fats become simpler in the order: aquatic animals ⟶ amphibia ⟶ reptiles ⟶ birds and rodents ⟶ land animals. The effect of diet and other circumstances on the composition of animal fats is discussed, and a general survey of the animal fats so far analysed indicates that there is a correlation between I.V. of the fat and fatty acid composition. Expressions have been derived for fats of I.V. less than 90 which will give the approximate composition of the fat, using the I.V. only.

Part II - Separation and Identification of Unsaturated Acids

A number of animal fats are characterised by complex mixtures of polyethenoid acids, and as yet no satisfactory

method has been evolved for separating and identifying them. Assuming that a polyethenoid acid could be separated free of all major impurities, the problem of determining its structure was discussed and a method providing unambiguous results was investigated. This method involved a system of partial hydroxylation and was successful in the case of methyl linoleate. Further development is required, however, before this method can be applied to other more unsaturated acids. In the course of this work a micromethod of analysing mixtures of mono-and dicarboxylic acids by partition chromatography was developed.

Several attempts were made to separate polyethenoid acids from a sample of crocodile fat in a sufficiently pure state for characterisation. Various techniques such as fractional crystallisation, fractional distillation, chromatography and formation of urea complexes were utilised, but none of them was entirely successful.

Part III - The Constitution and Properties of Santalbic Acid

Santalbic acid, the chief component acid in *Santalum album* (Linn.) seed oil is shown to be *trans*-octadec-11-en-9-ynoic acid, and thus identical with ximenynic acid. Some reactions of the acid and its derivatives are investigated.

A portion of this work has been published in three papers (*Biochem. J.*, 57, 459, 462; *J. Chem. Soc.*, in the press) and the publication of further papers is envisaged.

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Part I Analysis of Animal Fats

Introduction

Analyses of vegetable fats have covered a sufficiently wide range of species to indicate that there is a correlation between seed fat composition and botanical characteristics. However, among the fats of land animals there is a paucity of information and as yet only very tentative generalisations have been made. This position has been noted by several workers (e.g. Gunstone and Paton 1953) [For references in Part I see page 125] and the need for further analyses in this field has been stressed. Thus, Barker and Hilditch (1950) state:- "Attention is drawn to the interesting similarities and differences in animal fat composition in order to stress the need for many more data on this subject from a wide variety of species, and in the hope that others may be attracted to engage in similar studies with the aid of the experimental methods which are now available for the determination of the component acids of natural fats with considerable precision." These authors further list the land animal fats which they consider to have been satisfactorily analysed at that time. These are lion, Ceylon bear, giant panda, baboon, emu, kangaroo, tiger, puma, black bear, some species of deer and a relatively large number of analyses of the depot fats of oxen, sheep and pigs. Analyses have since been reported for hippopotamus (Barker and Hilditch 1950),

badger (Gupta, Hilditch and Meara 1950), horse (Brooker and Shorland 1950; Gupta and Hilditch 1951; Shorland, Bruce and Jessop 1952), rabbit (Clément and Meara 1951, Shorland 1953) and tiger (Pathak and Agarwal 1952). In the amphibian and reptile class Barker and Hilditch name frog, tortoise, lizard and turtle; recent analyses have added toad (Cattaneo et al 1951), moccasin (Pollard and McLaughlin 1952), python and diamond snake (Karkhanis and Magar, 1955), and crocodile (Pathak and Pande 1955). This obvious gap in our knowledge of the composition of animal fats has led to the initiation in this department of a series of researches of which the present work is a continuation (see Paton 1953). Analyses of the following animal fats have been completed in respect of this scheme:- deer and camel (Gunstone and Paton 1953a); python (Gunstone and Paton 1953b); chimpanzee (Gunstone 1955a); tiger and puma (Gunstone 1955b); crocodile (Gunstone and Russell 1954a); ostrich (Gunstone and Russell 1954b); mouse; rabbit; porcupine; flamingo and antelope. The present work describes the analyses of the last seven fats. A sample of seal oil was also investigated in anticipation of further work on the more unsaturated fatty acids present in marine oils.

The importance of vegetable oils in commerce has undoubtedly stimulated the relatively large series of investigations into the component acids of seed oils. The

fats of the less common animals, have obviously little commercial importance and this fact coupled with the difficulty of obtaining samples from their natural sources in a condition which could be utilised commercially or analytically probably explains the lack of data in this field. This latter difficulty can be overcome to a certain extent as Hilditch (1947a) suggests, by co-operation with the zoological authorities. Although the diet and environment of the animal are almost certainly not those of its natural habitat, and there is the further complication that the animal has probably died of disease and/or senility, results of considerable comparative value can be obtained. Thus the analyses in this work with one exception have been carried out on animals which have been living in zoos or similar institutions.

Method of Analysis

The fundamental feature of fat analysis is the determination of the composition of the mixed fatty acids produced on hydrolysis of the fat. This requirement involves the separation of the mixed acids into fractions containing not more than two saturated acids, accompanied by the corresponding unsaturated acids. This may be accomplished by a dual process of fractional crystallisation of the acids and fractional distillation of their methyl esters through an efficient column. In this way fractions may be obtained which satisfy the above conditions and the composition of each may then be determined from the appropriate characteristics viz. weight, saponification equivalent (giving a measure of the mean molecular weight), iodine value (giving a measure of the mean unsaturation) and absorption coefficient ($E^{1\%}_{1cm}$) after isomerisation.

Hydrolysis

If enough fat is available about 200gm. should be used. This amount is necessary to obtain reliable results for the minor component acids. However it is obvious that it will be impossible to obtain such a relatively large quantity of fat from smaller animals, and in most of the analyses in this work the amount of fat used was much smaller. To obtain enough material it is often necessary to take samples from different parts of the animal and the result obtained is

thus an average analysis, since composition may vary with locality in the body (cf. Hilditch 1947b: Winter and Nunn 1950). Then again, in the case of the very small animals enough fat can only be acquired by extraction from a number of different animals and accordingly only an average analysis is obtained. Thus a sample of mouse fat weighing 60gm. was obtained from 80 animals.

The hydrolysis is carried out by refluxing the fat for about 1 hour in ethanolic potassium hydroxide solution (5 ml. of ethanol per gm. of fat containing $\frac{1}{3}$ of weight of fat of potassium hydroxide dissolved in a small quantity of water). About one third of the alcohol is then distilled off, plenty of water added and the solution acidified with 25% sulphuric acid to precipitate the mixed acids. These acids on cooling are extracted with ether and the extract washed with water until free of sulphuric acid. Drying of the ethereal solution and removal of the solvent give the mixed acids.

Fractional Crystallisation of the Mixed Acids

This procedure is an important step in the analysis of fats, since an efficient separation at this stage is necessary for obtaining simpler fractions in the later stages of the analysis. This crystallisation, if successful, can divide the mixed acids into three fractions consisting of mainly saturated (A fraction), monoethenoid (B fraction) and polyethenoid acids (C fraction). Where a wide range of

acids are present this simple division is not achieved e.g. the lower saturated acids tend to crystallise with the polyethenoid (C) fraction. Nevertheless an efficient separation can often be obtained e.g. 55.0gm. of mouse fat mixed acids gave 15.1gm. of A fraction (I.V. 1.5), 21.7gm. of B fraction (I.V. 97.5) and 18.2gm. of C fraction (I.V. 159.6).

This is achieved by dissolving the acids in methanol (10c.c. per gm. of acids) and allowing the solution to crystallise at a temperature of about -55°C overnight (Gunstone and Paton 1953a). This temperature was varied depending on the unsaturation of the fat - in general, the more unsaturated ones are crystallised at lower temperatures at this stage. The crystallisation was carried out in a large Dewar flask containing about 7 litres of alcohol cooled a few degrees below the desired temperature with solid carbon dioxide. Under these conditions and by carefully insulating the top of the flask the temperature only rises a few degrees overnight. The filtration of the solids deposited was executed in a specially adapted Buchner funnel which was cooled by a bath containing alcohol and solid carbon dioxide a few degrees below the temperature of crystallisation (see Paton 1953). Removal of the solvent from the filtrate gave the polyethenoid C fraction and an intermediate fraction can be obtained from the solids by dissolving in methanol

and subsequent removal of the solvent. This latter fraction was recrystallised by dissolving in methanol (10c.c. per gm. of acids) and placing in a refrigerator at -20°C. A similar filtration gave two fractions - the monoethenoid (B) fraction from the filtrate and the saturated (A) fraction from the solids. Determinations of the iodine values were made at all stages to check the progress of the separation.

Esterification

The more unsaturated fractions (i.e. B and C) were esterified by dissolving in methanol (5c.c. per gm. of acids), passing in dry hydrogen chloride (1 gm. per 100c.c. methanol) and leaving the resultant solution overnight. About one third of the alcohol was then removed under reduced pressure at about 40°C and the esters were extracted with ether, washed with potassium hydroxide solution to remove any unesterified acid and the ether solution, after washing free of alkali with water, dried with anhydrous magnesium sulphate. Removal of the solvent gave the methyl esters in good yield. In the case of the most saturated A fraction treatment at a higher temperature was required since both the acids and their esters are largely insoluble in methanol at room temperature. The acids were thus dissolved in methanol as usual, concentrated sulphuric acid added (1% of weight) and the solution refluxed for about 1 hour. After removal of some of the alcohol the esters were extracted as usual.

Fractional Distillation of the Methyl Esters

The estimation and identification of minor component acids require an efficient fractionation of the methyl esters. A number of columns have been used in this type of work e.g. the spinning band column (Winter and Nunn 1953) which is reputed to be very efficient. In this department the column available was a Towers (T. 117) electrically heated column, packed with glass helices. Distillation of the methyl esters under reduced pressure on this column gave satisfactory results. Fractions of about 3gm. were collected, as this quantity gave the most satisfactory determinations of saponification equivalents. The fractions were collected in a special multiple receiver (see Paton 1953). However in some of the analyses it was found necessary to collect smaller fractions in which case a few minor modifications had to be made (e.g. flamingo fat, fraction C). The saponification equivalent and iodine value of each of the fractions obtained by distillation were determined.

Unsaponifiable Matter

When the unsaponifiable matter exceeds 1 or 2% of the whole fat, it is best removed by continuous ether extraction of the aqueous alcoholic soap solution obtained after saponification of the fat and before the precipitation of the acids (Hilditch 1947c.). In the present samples however this was not the case and the unsaponifiable matter generally

concentrated in the residues from the fractional distillation. This was determined quantitatively on the acids remaining after determination of the saponification equivalent. The acids were converted to their potassium salts and the unsaponifiable matter extracted with ether under standard conditions (Sub-Com. on Det$^{n.}$ of Unsap. Matter in Oils and Fats 1933) and weighed directly. The necessary corrections were then made to the constants of the residual fractions and the composition computed as usual.

Determination of the Polyethenoid Acids

In the case of these fractions which evidently contain saturated, mono and polyethenoid acids, the composition cannot be ascertained from the weight, saponification equivalent and iodine value only - some other characteristic is required. Older methods make use of the thiocyanogen value, elaidinisation and bromo addition compounds, but these are not very accurate. The best method, now widely used is that based on the measurement of the ultraviolet absorption of the polyethenoid acids after isomerisation in alkali. The first quantitative method using this procedure was proposed by Mitchell, Kraybill and Zscheile (1943) and later modified by other workers (e.g. Hilditch, Morton and Riley 1945; Hilditch, Patel and Riley 1951). The method as adapted by these workers is mainly used for determining linoleic and linolenic acids separately or together and depends on the fact that a solution of potassium hydroxide in ethylene glycol at high temperatures

is capable of isomerising linoleic acid, which contains two double bonds separated by a methylene group, into the conjugated form. This is true of linolenic and other similar polyethenoid acids. The isomerisation always takes place to the same extent when carried out under standard conditions, and the amounts of conjugated acids can be determined by spectroscopic means i.e. the standard conditions for the determination of linoleic acid are to isomerise with 7½% solution of potassium hydroxide in ethylene glycol at 180°C for 60 minutes, whereas linolenic acid is isomerised at 170°C for 15 minutes with the same reagent. Other conditions are used for the determination of tetra-, penta- and hexaenoic acids (see Herb 1955). The constants used in the above procedures were determined on acids derived from vegetable sources, and while there is no conclusive evidence to suggest that the acids from animal fats are essentially of the same nature, the method gives results which are consistent with other observed values such as iodine values. Several attempts were made to elucidate the structure of these polyethenoid acids (e.g. see analysis of crocodile fat page 24, analysis of ostrich fat page [34] and part II).

Method of Calculation

It is difficult to make any generalisations regarding the method of calculation as each fraction has to be considered

in relation to its saponification equivalent (S.E.), iodine value (I.V.) and absorption coefficient ($E^{1\%}_{1cm}$) after alkali isomerisation. Accordingly the scheme of calculation can best be illustrated by considering one fat in detail, and this is done by examining the results of the crocodile analysis.

The method of calculation is essentially that described by Hilditch (1947d). It is convenient at this stage to indicate the convention regarding the nomenclature of the fatty acids and esters. Thus esters of acids containing 18 carbon atoms are referred to as C_{18} esters and esters of mono, di and trienoic C_{18} acids are referred to as $C_{18}{}^{1}$, C_{18}ll and C_{18}lll esters respectively. Further, saturated acids are given the superscript O - in this way palmitic acid would be $C_{16}{}^{\circ}$.

Use is also made of the expressions 'C_{16}' or 'C_{18}', these implying a mixture of C_{16} or C_{18} acids or esters.

Fraction A (See Table 1)

On examination of the I.V. and S.E. of the first twelve fractions it is obvious that this fraction consists mainly of C_{16} esters (e.g. S.E. of $C_{16}{}^{\circ}$ ester is 270.4). It is assumed that the small iodine values obtained in the fractions A1-A10 are due to C_{16}l esters and that the I.V. in the case of A11-A16 is due to unsaturated C_{18} esters. Thus in the case of A6 the weight of unsaturated ester ($C_{16}{}^{1}$) is given by

wt. of fraction x $\frac{\text{I.V. of fraction}}{\text{I.V. of pure unsaturated ester } (C_{16}{}^{1})}$

i.e. $C_{16}{}^{1} = 3.53 \times \frac{0.8}{94.6} = 0.03$. The remainder of A6 will consist of $C_{16}{}^{\circ}$. Fractions A3-A10 can be calculated in a similar way. In fractions A1 and A2 it is evident from the lower S.E.'s that acids of shorter chain length are present. It is assumed that these fractions contain esters of $C_{14}{}^{\circ}$, $C_{16}{}^{\circ}$ and $C_{16}{}^{1}$. The weight of $C_{16}{}^{1}$ is calculated as above; the S.E. of the remaining saturated esters is corrected to allow for the removal of the $C_{16}{}^{1}$ and the saturated part is partitioned between myristic and palmitic esters according to the corrected S.E.'s. The stages are therefore for A1, wt. of unsaturated ester $(C_{16}{}^{1}) = 1.1 \times \frac{2.64}{94.6} = 0.03$ giving the weight of saturated esters as 2.61. It then follows, from the definition of S.E. that $\frac{2.64}{258.4} = \frac{2.61}{\text{S.E. of satd. esters}} + \frac{0.03}{268.4}$ i.e. S.E. of sat'd. esters = 258.3, and if x is % of C_{16} esters present in the saturated part then

$$\frac{x}{270.4} + \frac{100-x}{242.4} = \frac{100}{258.3} \quad \text{i.e. } x = 59.55\%.$$

This gives the composition of the fraction as $C_{16}{}^{1}$ 0.03: $C_{16}{}^{\circ}$ 1.55: $C_{14}{}^{\circ}$ 1.06.

Table 1 - Distillation of A esters of C. porosus

Fraction	wt. gm.	I.V.	S.E.	C_{14}°	C_{16}°	C_{18}°	C_{20}°	C_{16}'	'C_{18}'	N.S.+
1	2.64	1.1	258.4	1.06	1.55	-	-	0.03	-	-
2	2.71	1.2	268.8	0.14	2.54	-	-	0.03	-	-
3	2.80	1.0	270.1	-	2.77	-	-	0.03	-	-
4	3.22	0.8	270.6	-	3.19	-	-	0.03	-	-
5	3.32	0.6	270.6	-	3.30	-	-	0.02	-	-
6	3.53	0.8	270.0	-	3.50	-	-	0.03		
7	3.08	0.5	270.2	-	3.06	-	-	0.02	-	-
8	3.41	0.7	270.1	-	3.38	-	-	0.03	-	-
9	3.40	0.3	270.0	-	3.39	-	-	0.01	-	-
10	3.06	0.6	270.3	-	3.04	-	-	0.02	-	-
11	3.34	1.8	270.5	-	3.27	-	-	-	0.07	-
12	2.92	24.7	282.7	-	1.52	0.62	-	-	0.78	-
13	3.23	41.0	295.9	-	0.17	1.63	-	-	1.43	-
14	3.12	34.9	296.3	-	0.14	1.80	-	-	1.18	-
15	2.42	27.2	295.7	-	0.17	1.54	-	-	0.71	-
16	3.00	22.3	309.3	-	-	1.42	0.82	-	0.72	0.04
Total	49.20			1.20	34.99	7.01	0.82	0.25	4.89	0.04
% Esters				2.44	71.11	14.25	1.67	0.51	9.94	0.08
% Acids				2.42	71.02	14.30	1.69	0.51	9.98	0.08
% Fraction (33.2)				0.80	23.58	4.75	0.56	0.17	3.31*	0.03

* Using isomerisation data this gives 3.06 C_{18}^{1}, 0.25 C_{18}^{11}.

\+ Unsaponifiable matter.

Isomerisation Data

A13 after isomerisation at 180°C/60min. had $E_{1cm}^{1\%}$ of 31.1 at 234mμ.

The composition of A2 can be computed in a similar manner. From the S.E.'s and I.V.'s of A12-A15 it is assumed that they contain C_{16}°, C_{18}° and unsaturated C_{18} esters. i.e. two saturated and one unsaturated ester as above and hence the results can be calculated similarly. This A fraction is not very typical because according to spectroscopic data these later fractions do contain a little octadecadienoic acid which can be calculated as shown below for the B fraction. The residue (A16) contains a little unsaponifiable material and taking account of this the S.E. becomes 305.2 and the I.V. 22.6. Once more the I.V. is assumed to be due to 'C_{18}' esters and the remaining saturated esters are partitioned between C_{20}° and C_{18}° as in A1.

Fraction B. (See Table 2)

This fraction is the intermediate one and it is clear that it will contain a fair proportion of unsaturated esters, and as the isomerisation data in Table 2 show these esters are mainly monounsaturated. This last statement is justified by the observation (Hilditch, Patel and Riley 1951) that under the standard conditions of isomerisation pure linoleic acid gives a maximum absorption at 234mμ with $E_{1cm}^{1\%}$ of 906 and pure linolenic acid shows maximum absorption at 268mμ with $E_{1cm}^{1\%}$ of 555.

Considering B9 as containing only C_{18} esters (from S.E.),

from the spectral values, % $C_{18}{}^{11} = \frac{64.0 \times 100}{906} = 7.1$ and since the I.V. of pure $C_{18}{}^{11}$ ester is 173.2, the contribution of this $C_{18}{}^{11}$ to the I.V. of the whole fraction is

$\frac{7.1 \times 173.2}{100} = 12.2$ i.e. % $C_{18}{}^{1} = \frac{(92.5 - 12.2) \times 100}{85.6} = 93.8\%$

(since I.V. of pure $C_{18}{}^{1}$ is 85.6). It will thus be seen that $C_{18}{}^{1} + C_{18}{}^{11} = 100.9\%$ and it is assumed that this fraction and similar ones (i.e. B5 - B14) contain $C_{18}{}^{1}$ and $C_{18}{}^{11}$ only i.e. two unsaturated esters and hence the composition of these fractions can be computed using the I.V. only.

i.e. wt. of $C_{18}{}^{11} = \left(\frac{\text{I.V. of fraction} - 85.6}{173.2 - 85.6}\right)$ x wt. of fraction

The constants for B3 indicate that this fraction contains some esters lower than the C_{18} series. It is assumed that this contains a mixture of C_{16} and C_{18} esters and the latter are taken to have the same composition as in B5 (the earliest fraction containing C_{18} only) i.e. C_{18} esters have I.V. of 92.2 and S.E. (calculated) of 296.3. By an approximate calculation ignoring the correct unsaturations of the fractions it can be shown that the S.E. of the C_{16} esters should be approximately 269.6. Thus assuming that the S.E. of the C_{16} ester is 269.6.

$$\frac{100 - C_{18}}{269.6} + \frac{C_{18}}{296.3} = \frac{100}{273.6} \quad \ldots (A)$$ i.e. $C_{18} = 16.1\%$

$\therefore$ I.V. due to 'C_{18}' = 14.8 and I.V. due to 'C_{16}' = 46.1-14.8 = 31.3

i.e. I.V. of C_{16} esters is 37.3 whence S.E. is 269.6.

This implies that the estimated equivalent (269.6) is in fact correct and that the acids must indeed be present in the above proportions i.e. C_{18} = 16.1%. (If however, the value for the S.E. is not the same as originally assumed then the latter value is substituted in (A) and the process repeated until similar values are obtained). It follows that the contribution of C_{18}^{11} to the $E^{1\%}_{1cm}$ observed for this fraction is $\frac{0.25 \times 906 \times 16.1}{3.37 \times 100}$ = 10.8. Since the observed value was 16.4 and in view of the uncertainty of the nature of the octadecadienoic acids and the C_{16} unsaturated acids the difference between the observed reading and the value calculated for C_{18}^{11} can be neglected i.e. there is no evidence of the presence of C_{16}^{11} esters and it is assumed that the I.V. of the C_{16} esters is due entirely to the presence of C_{16}^{1} esters i.e. % $C_{16}^{1} = \frac{37.3 \times 100}{94.6}$ = 39.4. Thus, the composition of B3 is C_{18}^{1} - 0.49; C_{18}^{11} - 0.04; C_{16}° - 1.68; C_{16}^{1} - 1.09. Fraction B4 is partitioned between C_{16} having the composition as found in B3 and C_{18} as in B5 on the basis of I.V. i.e. 'C_{16}' = $\frac{92.2 - 84.4}{92.2 - 37.3} \times 3.18 = 0.45$. In B2, it is obvious from the S.E. that some C_{14} esters are present. The supposition is therefore made that this fraction contains C_{14}°, C_{14}^{1} and C_{16} esters as in B3.

Table 2 - Distillation of B esters of C. porosus

Fraction	wt. gm.	I.V.	S.E.	C_{14}°	C_{16}°	C_{14}^{1}	C_{16}^{1}	C_{18}^{1}	C_{18}^{II}	'C20'	N.S.
1	2.41	7.8	255.9	0.99	1.24	0.18	-	-	-	-	
2	2.56	34.5	262.6	0.46	1.19	0.14	0.77	-	-	-	
3	3.30	46.1	273.6	-	1.68	-	1.09	0.49	0.04	-	
4	3.18	84.4	293.4	-	0.27	-	0.18	2.53	0.20	-	
5	3.37	92.2	296.5	-	-	-	-	3.12	0.25	-	
6	3.20	92.4	297.3	-	-	-	-	2.95	0.25	-	
7	3.24	92.8	296.2	-	-	-	-	2.97	0.27	-	
8	3.44	92.4	295.4	-	-	-	-	3.17	0.27	-	
9	3.39	92.5	296.4	-	-	-	-	3.12	0.27	-	
10	3.04	91.8	296.2	-	-	-	-	2.82	0.22	-	
11	3.29	91.1	295.2	-	-	-	-	3.08	0.21	-	
12	3.28	90.7	295.5	-	-	-	-	3.09	0.19	-	
13	3.46	90.0	296.3	-	-	-	-	3.29	0.17	-	
14	2.83	89.9	295.8	-	-	-	-	2.69	0.14	-	
15	2.02	89.3	329.3	-	-	-	-	-	-	2.02	
16	2.70	109.6	316.5	-	-	-	-	-	-	2.58	0.12
Total	48.71			1.45	4.38	0.32	2.04	33.32	2.48	4.60	0.12
% Esters				2.98	8.99	0.66	4.19	68.40	5.09	9.44	0.25
% Acids				2.95	8.95	0.65	4.17	68.44	5.09	9.49	0.26
% Fraction (28.1)				0.83	2.51	0.18	1.17	19.24	1.43	2.67	0.07

Isomerisation Data

Fraction	I.V. of acids	$E^{1\%}_{1cm}$ at 234mμ (after isomerisation at 180°C/60 min.)
B15	92.6	42.6
B9	96.5	64.0
B3	44.9	16.4

U.V. absorption at 268mμ and of unisomerised fractions was negligible.

It can be shown that for a tertiary mixture of two unsaturated esters [Higher (H) and Lower (L)] and a saturated ester (S) that

$$H = \frac{W[I.V._W X + I.V._L(-Z)]}{[I.V_W X + I.V._L(-Y)]}$$

where $X = R_L - R_S$

$Y = R_H - R_S$

$Z = R_W - R_S$

W refers to the whole fraction and in particular to its weight; R is the reciprocal S.E. In this case 'C_{16}' = H, $C_{14}{}^{1}$ = L and $C_{14}{}^{c}$ = S and the composition of B2 can be readily computed. Fraction B1 by the above method gives a negative value for H - the fraction is therefore assumed to consist of $C_{14}{}^{1}$, $C_{14}{}^{c}$ and $C_{16}{}^{c}$ and the results calculated as in A1. Fraction B15 is assumed to consist of 'C_{20}' only and the average unsaturation of the esters can be ascertained from the I.V. The assumption is also made that the residual fraction B16 consists only of 'C_{20}' and the unsaponifiable matter.

Fraction C (See Table 3)

The same kind of procedure was applied to this fraction although it is evident that it contains more unsaturated acids than the B fraction, and further that the acids cover a wider range (i.e. C_{12} - C_{20}). Considering fraction C9 from S.E. it is likely to consist of C_{18} only.

Using spectral values $C_{18}^{111} = \frac{49.9 \times 100}{555} = 9.0\%$

$$C_{18}^{11} = \frac{529 - 51.7}{906} \times 100 = 52.6\%$$

These acids contribute 119.8 (24.6 + 95.2) to the I.V. of the acids i.e. the I.V. due to $C_{18}^{1} = 33.2$ whence $C_{18}^{1} = 37.0\%$. It will be seen that this gives a total of 98.6% for these acids (i.e. $C_{18}^{1} + C_{18}^{11} + C_{18}^{111}$) and it seems reasonable to assume that these are the only ones present in the fraction i.e. C_{18}^{111} 9.0%; C_{18}^{11} 52.6%; C_{18}^{1} 38.4%. This composition is used for fractions C6 - C14 which have similar S.E.'s and I.V.'s. C4 is considered to be a mixture of C_{16} and C_{18} (as in C6) and the results are computed in a similar manner to those of B3. Fraction C5 is partitioned between C_{16} (as in C4) and C_{18} (as in C6) and the results calculated as in B4.

Table 3 - Distillation of C Esters of C. porosus

Fraction	wt. gm.	I.V.	S.E.	C_{12}°	C_{14}°	C_{16}°	C_{12}^{1}
1	2.64	37.2	232.4	0.57	1.17	-	0.23
2	2.95	84.0	261.8	-	0.33	0.17	-
3	2.53	91.1	265.6	-	0.07	0.18	-
4	2.54	100.6	272.0	-	-	0.17	-
5	2.86	138.6	287.9	-	-	0.03	-
6	2.87	147.5	292.4	-	-	-	-
7	2.93	148.8	292.4	-	-	-	-
8	3.16	148.8	292.2	-	-	-	-
9	2.98	148.6	291.6	-	-	-	-
10	2.77	149.3	291.8	-	-	-	-
11	3.01	147.5	291.8	-	-	-	-
12	3.52	148.1	291.9	-	-	-	-
13	3.69	145.8	291.7	-	-	-	-
14	3.11	146.9	291.3	-	-	-	-
15	2.34	165.9	296.0	-	-	-	-
16	5.60	173.6	308	-	-	-	-
Totals	49.50			0.57	1.57	0.55	0.23
% Esters				1.15	3.17	1.11	0.46
% Acids				1.13	3.14	1.10	0.45
% Fraction (38.7)				0.44	1.22	0.43	0.17

Isomerisation Data

Fraction	I.V. of acids	$E_{1cm}^{1\%}$ at 234mμ*	$E_{1cm}^{1\%}$ at 268 mμ+
C4	105.1	125.7	-
C9	153.0	529.0	49.9
C15	173.0	386.0	178.9

* after isomerisation at 180°C/60 min.

\+ after isomerisation at 170°C/15 min.

U.V. absorption of unisomerised fractions was negligible.

C_{14}^{1}	C_{16}^{1}	C_{16}^{11}	C_{18}^{1}	C_{18}^{11}	C_{18}^{111}	'C_{20}'	N.S.
0.67	-	-	-	-	-	-	-
0.34	1.98	0.13	-	-	-	-	-
0.16	1.99	0.13	-	-	-	-	-
-	1.88	0.12	0.14	0.20	0.03	-	-
-	0.40	0.03	0.92	1.26	0.22	-	-
-	-	-	1.10	1.51	0.26	-	-
-	-	-	1.13	1.54	0.26	-	-
-	-	-	1.21	1.67	0.28	-	-
-	-	-	1.14	1.57	0.27	-	-
-	-	-	1.06	1.46	0.25	-	-
-	-	-	1.16	1.58	0.27	-	-
-	-	-	1.35	1.85	0.32	-	-
-	-	-	1.42	1.94	0.33	-	-
-	-	-	1.19	1.64	0.28	-	-
-	-	-	2.42	3.31	0.57	1.37	0.27
-	-	-					
1.17	6.25	0.41	14.24	19.53	3.34	1.37	0.27
2.36	12.63	0.83	28.77	39.45	6.75	2.77	0.55
2.33	12.59	0.83	28.82	39.49	6.75	2.79	0.58
0.90	4.87	0.32	11.15	15.29	2.61	1.08	0.22

C2 and C3 are assumed to contain C_{14}^{0}, C_{14}^{1} and 'C_{16}' (as in C4), the composition of these fractions being calculated using the formula as described for B2. Fraction C1 is considered as C_{12}^{0}, C_{12}^{1}, C_{14}^{0} and C_{14}^{1} i.e. it contains two saturated and two unsaturated esters; then only a small error is entailed by taking the equivalent of the unsaturated part as the same as that as the fraction itself (cf. Hilditch 1947d)

$$\text{i.e.}\frac{C_{12}^{1}}{\text{S.E. of } C_{12}^{1}} + \frac{C_{14}^{1}}{\text{S.E. of } C_{14}^{1}} = \frac{C_{12}^{1} + C_{14}^{1}}{\text{SEobs.}} \quad \ldots\ldots \text{(A)}$$

Further

$$C_{12}^{1} \times \text{I.V. of } C_{12}^{1} + C_{14}^{1} \times \text{I.V. of } C_{14}^{1} = 100 \times \text{I.V. obs.} \quad \text{(B)}$$

Solving (A) and (B) simultaneously gives values for C_{12}^{1} and C_{14}^{1} and the remaining part can be partitioned between the two saturated esters in the usual way. Fractions C15 and C16 were combined for calculation and the esters were considered to consist of 'C_{18}' as in C14 and 'C_{20}' - the composition was then computed as in B3.

The esters were then totalled as shown in the tables and converted to '% acids' and finally to increments of the totalled mixed acids expressed as % wt. These increments were totalled and then expressed as % wt. and % mol. excluding unsaponifiable as in Table 4.

Table 4 - Component Acids of C. porosus fat

Acid	A	B	C	Total	Excluding Unsaponifiable % wt.	Excluding Unsaponifiable % mol.
Saturated						
Lauric	-	-	0.44	0.44	0.44	0.59
Myristic	0.80	0.83	1.22	2.85	2.86	3.39
Palmitic	23.58	2.51	0.43	26.52	26.61	28.10
Stearic	4.75	-	-	4.75	4.77	4.54
Arachidic	0.56	-	-	0.56	0.56	0.49
Unsaturated						
Dodecenoic	-	-	0.17	0.17	0.17	0.23
Tetradecenoic	-	0.18	0.90	1.08	1.08	1.29
Hexadecenoic	0.17	1.17	4.87	6.21	6.23	6.63
Hexadecadienoic	-	-	0.32	0.32	0.32	0.34
Octadecenoic	3.06	19.24	11.15	33.45	33.56	32.16
Octadecadienoic	0.25	1.43	15.29	16.97	17.02	16.43
Octadecatrienoic	-	-	2.61	2.61	2.62	2.55
As eicosenoic+	-	2.67	1.08	3.75	3.76	3.26
Unsaponifiable	0.03	0.07	0.22	0.32	-	-

+ average unsaturation - 3.5H.

The Analysis of Crocodile Fat

Introduction

The order Crocodilia comprises the crocodiles proper together with alligators and gharials. The analytical record regarding fats of this order is particularly barren and until recently the only figures available were those from an African crocodile and the related alligator obtained by Kobayshi (1922) and from samples of alligator oil (Luhr 1932, Nembrot and Cadrobbi 1936). These accounts, however, merely record the iodine values and other constants and the authors make no attempt to carry out a detailed analysis. The Colonial Products Advisory Bureau (Plant and Animal) has recently shown an interest in crocodile oil and carried out a simple examination on samples of oil from Tanganyika, primarily with a view to its possible market value (Bennet, Brown, Coomes, Morton and Raymond 1950). These authors have suggested that crocodile oil might find a use alongside turtle oil in cosmetic preparations. Since it is uncertain why turtle oil is favoured in this connection it is difficult to say how far the present results add to what is known about crocodile oil relative to its use in this way.

After this work had been published (Gunstone and Russell 1954a) an analysis of an Indian crocodile fat (Gavialus gangeticus) was reported (Pathak and Pande 1955).

Origin and Preparation of the Fat

Two samples of fat were investigated. One was obtained from an adult Estuarine crocodile (*Crocodylus porosus*) of about 20 years which had been kept in captivity in Edinburgh Zoo where its diet consisted of whole dead piglets. A post mortem examination showed some enteritis and a fatty infiltration of the liver. The crude fatty material after autoclaving at 120°C was broken up in a homogeniser and extracted with light petroleum (b.p. 40-60°C) giving a pale yellow semi-solid fat (for details see Paton 1953). The second sample of fat, obtained through the kindness of the staff of the Colonial Products Advisory Bureau, came from a crocodile (*Crocodylus niloticus*) in Tanganyika living presumably in its natural state.

These fats were analysed in the manner already indicated. The detailed results are given in the Appendix page 86 .

Examination of Unsaturated Acids of C.porosus and C. niloticus fats

Unsaturated C_{16} acids

Evidence for the presence of C_{16} polyethenoid acids is derived only from spectroscopic data and it seems probable that these acids are a complex mixture (for a further discussion on this subject see p. 34 where a similar circumstance occurs in the unsaturated acids of ostrich fat).

The occurrence of hexadec-9-enoic acid was shown by oxidation with alkaline potassium permanganate (Lapworth and Mottram 1925) to give 9:10 dihydroxypalmitic acid (m.pt. 123-124°C from C. porosus and m.pt. 126-127°C from C. niloticus

Unsaturated C_{18} acids

It has long been recognised that the yield of a bromo derivative from any given polyethenoid acid is not quantitative; however by carrying out the bromination and separation of the bromides in a rigid and prescribed manner certain empirical values have been obtained which have been designated polybromide numbers. By means of suitable equations these polybromide numbers can be related to the percentage of the individual components of a given mixture of unsaturated acids, although it should be recognised that in certain cases considerable errors may arise because of mutual solubility effects which the bromo derivatives exert on each other (Markley 1947). This method depends essentially on the separation of the ether-insoluble hexabromides from the tetrabromides which are soluble in ether and insoluble in petroleum ether (b.p. 40-60°C).

Bromination of a fraction from C. porosus rich in C_{18} polyethenoid acids (C9) was effected quantitatively and using constants applicable to linolenic and linoleic acids results were obtained as shown in Table 5. These agree fairly well with the values obtained by the isomerisation

procedure and suggest that the octadecadi- and trienoic acids of *C. porosus* are substantially the all *cis* isomers as found in vegetable oils.

Table 5 - Polyethenoid C_{18} acids

	Bromination		Alkali isomerisation	
	Diene	Triene	Diene	Triene
C. porosus	48.7	12.9	44.4	14.3
C. niloticus	16.9	17.9	12.8	23.8

Values are given as % wt. of fractions containing only C_{18} acids.

This conclusion is confirmed by the additional spectrographic data as recorded in Figure 1. Jackson, Paschke, Tolberg, Boyd and Wheeler (1952) in a study of the alkali isomerisation of linoleic acid and some of its geometrical isomers determined the period of isomerisation required to give the maximum U.V. absorption. This was found to be 45 minutes for the *cis*, *cis* acid (i.e. linoleic), 360 minutes for the *trans*, *trans* acid and 150 min. for the *cis*, *trans* acid. The detailed results given by these authors clearly shows that the *cis*, *cis* acid isomerises quicker than its geometrical isomers and since in our experiments the maximum value was observed after one hour, this can be taken as additional evidence that the octadecadienoic acid is essentially the *cis*, *cis* compound.

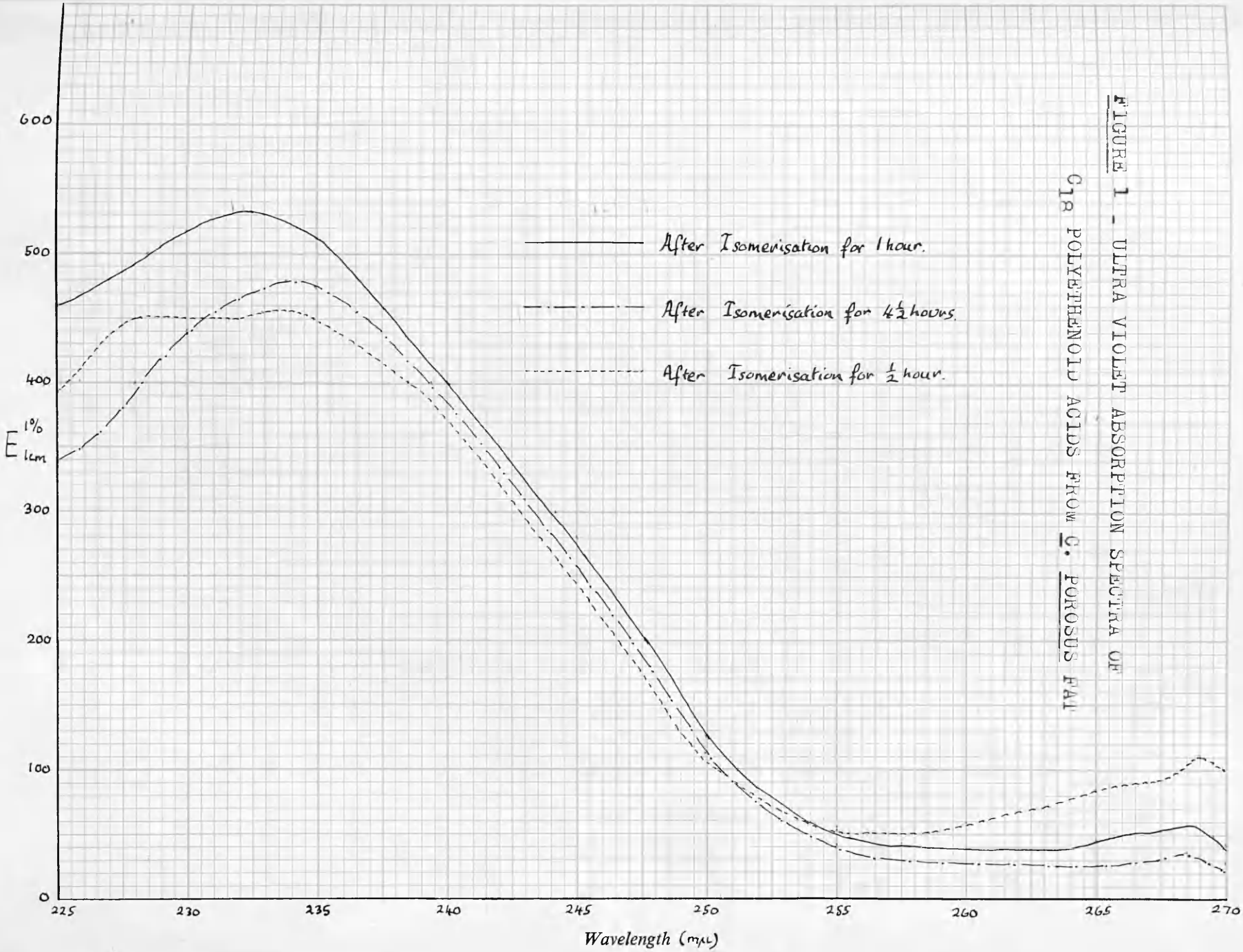


FIGURE 1 - ULTRA VIOLET ABSORPTION SPECTRA OF C_{18} POLYETHENOID ACIDS FROM C. POROSUS FAT

The results obtained in the bromination of C. niloticus (see Table 5) are less definite and although linoleic and linolenic acids are present (see below) they are probably accompanied by isomeric compounds. Further evidence of the presence of linoleic and linolenic acids in both these fats is provided by the recrystallisation of the bromo-derivatives prepared as above to give compounds identical with those derived from the acids found in vegetable fats.

i.e. C. porosus gave hexabromostearic acid m.pt. 184-187°C
and tetrabromostearic acid m.pt. 112-114°C

C. niloticus gave hexabromostearic acid m.pt. 177-179°C
and tetrabromostearic acid m.pt. 110-112°C.

Mixed m.pts. with authentic samples gave no depressions. It is interesting to compare these observations with the results obtained for the analysis of toadfat (Cattaneo, deSutton and Penhos 1951). In this fat evidence was found of the existence of other octadecatrienoic acids differing from linolenic acid, although the presence of linoleic and linolenic acids was shown by the preparation of the usual bromoderivatives.

Oleic acid was shown to be present in both the samples of crocodile fat by the oxidation of suitable fractions by alkaline potassium permanganate. (Lapworth and Mottram 1925) giving 9:10 dihydroxystearic acid [m.pt. 131°C from C. porosus and m.pt. 128.5 - 129°C from C. niloticus].

Unsaturated C_{20-22} acids

These unsaturated acids are rather complex in character and in the analysis of C. porosus fat they appear in fractions B and C. That portion which is present in fraction B must be largely monoethenoid in view of its low iodine value (approximately 90), its small absorption at 234mμ (approx. 40) after alkali isomerisation, and the fact that the higher saturated acids do not appear in this fraction. The homologous acids in fraction C are more unsaturated and since they show appreciable absorption after alkali isomerisation at 234mμ, 268mμ, 300 and 315mμ, they must contain a tetraethenoid acid (probably arachidonic acid) accompanied possibly by less unsaturated C_{20} acids.

The higher unsaturated acids of C. niloticus fat are present in greater amounts and include C_{22} as well as C_{20} acids. Both occur almost entirely in fraction C and are highly unsaturated (iodine value 220-300). Alkali isomerisation of the C_{20} fraction gives appreciable absorption maxima at 234mμ, 268, 300, 315 and 346mμ showing that pentaethenoid acids are present (see Herb 1955). Fractions from C. niloticus fat rich in C_{20} and C_{22} acids were also brominated and the solid products recrystallised from a variety of solvents. Two compounds were obtained and these were probably hexabromoeicosanoic acid (decomposes at

255°C: found Br 61.4%, calculated for $C_{20}H_{34}O_2Br_6$, 61.0%) and decabromodocosanoic acid (melts with decomposition at 260°C: found Br 70.6%, calculated for $C_{22}H_{34}O_2Br_{10}$, 70.7%), thus indicating that eicosatrienoic and docosapentaenoic acids occur in the C_{20} and C_{22} fractions.

Details of other simpler acids isolated are given in the appendix (pages 87,91). A further detailed qualitative examination of the unsaturated acids of C. niloticus fat is made in Part II (page 130).

Discussion

Klenk (1933) and Klenk, Ditt and Diebold (1935) have drawn attention to the fact that the fatty acid composition of certain amphibian and reptile fats studied by them was intermediate between the fats of marine and land animals. The results obtained in this group of animals since these original observations do not alter this contention, and reference to Table 6 shows that the analyses of crocodile fat accord with this general classification of amphibians and reptiles.

In comparing the composition of the three crocodile fats, it should be pointed out that the two analyses of fat from wild animals (i.e. C. niloticus and Gavialis gangeticus) show striking similarities. The main differences are in the distribution of the C_{18} acids, the totals for which are however almost exactly the same. In the more saturated

fat (I.V. of G. gangeticus fat was 72.6) there is more stearic acid present and less unsaturated C_{18} acids (cf. Gunstone 1955b).

Table 6 - Component Acids of Amphibian and Reptile Fats
(% wt.)

Acid	Frog	Tortoise	Turtle (i)	Turtle (ii)
Lauric	-	-	13.3*	-
Myristic	4	1	10.6	6.6
Palmitic	11	14	17.0	21.8
Stearic	3	4	4.1	15.5
Arachidic	-	-	-	1.9
Tetradecenoic	-	-	1.3	3.5
Hexadecenoic	15	9	7.8	18.0
Octadecenoic Octadecadienoic Octadecatrienoic	52 (-2.5H)	65 (-2.4H)	39.6 (-2.2H)	31.4 (-3.7H)
'Eicosenoic' 'Docosenoic'	15 (-6H)	7 (-4H)	6.1 (-6.3H)	1.3 (-8.6H)

All values are taken from Hilditch (1947) apart from results for turtle (ii) Giral and Marquez (1948), python (Gunstone and Paton, 1953), toad (Cattaneo et al 1951) and crocodile. The crocodile results are from the fat of (i) Crocodylus porosus (ii) Crocodylus niloticus (iii) Gavialis gangeticus (Pathak and Pande 1955).

* Also 0.2% decanoic acid + also 0.2% dodecenoic acid.

Crocodile (i)	Crocodile (ii)	Crocodile (iii)	Lizard (i)	Lizard (ii)	Python	Toad
0.4	-	0.2	-	-	-	0.5
2.9	3.9	4.2	4	4	1.3	3.4
26.6	24.0	25.8	18	29	19.7	18.2
4.8	3.4	8.7	7	10	10.8	3.8
0.6	1.3	-	-	-	1.2	0.5
1.1+	1.0	2.0	-	-	0.5	1.2
6.5	15.0	11.6	10	12	3.9	13.1
33.5	30.8	35.5 (-30H)	56 (-24H)	40 (-2.7H)	47.0	57.9
17.0	6.5				10.7	
2.6	3.1				0.8	
3.8	6.8	12.0 (-5.3H)	5 (-5H)	5 (-5.5H)	4.1	1.4
-	4.2				-	

It is also evident that the fat from these animals living in their natural state contains a greater proportion of C_{16} and C_{20-22} unsaturated acids, whereas the fat from C. porosus contains more octadecadienoic acid which is more certainly linoleic acid than in the former case. The differences probably reflect differences in diet since the captive animal was fed on whole dead piglets, whilst crocodiles in their natural habitat are reported to eat mainly fish. A similar comparison can be made between the two lizards: the specimen (i) was a mature animal which had been kept in captivity for a number of years whilst lizard (ii) was a very young animal of the same species which had been killed in Ceylon. It is also interesting to note that in spite of its lower I.V., the fat from G. gangeticus is more marine in type than that from C. porosus, because of the presence of higher quantities of C_{16} and C_{20-22} unsaturated acids.

As regards the suggestion by Bennet et al. (1950) concerning the substitution of crocodile for turtle oil in the cosmetics industry, comparison with the figures obtained for turtle oil certainly indicate similiarities. Giral and co-workers (1948) have made an extensive study of Mexican turtle oils - four samples of oil from different species were analysed, and these were found to vary somewhat

among themselves. Comparison of the composition of the oil from Caretta caretta [turtle (ii) in Table 6] with that from C. niloticus (this animal being in its natural state and belonging to the species potentially available) shows a marked similarity apart from the greater proportions of unsaturated C_{20-22} unsaturated acids in the crocodile oil. Moreover the oil from this species of turtle is readily available in the Mexican market and is widely used in cosmetics (Giral and Marquez 1948). In the other samples of turtle fat examined the proportion of C_{20-22} acids is very similar (e.g. Chelone mydas) although the distribution of the saturated acids is somewhat different. It is reported that the usefulness of turtle oil in cosmetics is due to the presence of higher unsaturated acids (cf. Harry 1941, 1948); these occur in most amphibian and reptile fats and also in larger amounts in fish oils. There may be olfactory objections to the use of the latter, and whilst at present many amphibian and reptile fats are not easily available, crocodile fat if available may thus find a use in cosmetics.

The Analysis of Ostrich and Flamingo Fats

Introduction

There are very few detailed analyses of bird depot fats, only those of the domestic hen, grey goose and emu having been studied in sufficient detail. It is obvious of course that there will be difficulties in the way of obtaining a large enough sample of fat from such small animals as birds. This fact probably explains the lack of data in this field; the ones that have been examined being among the bigger members of this group.

A superficial examination of ostrich fat has been carried out by Vamvakas (1910) who however merely records the I.V. (71.1), S.E.(265.4) and some other constants.

Origin and Preparation of the Fats

The ostrich fat used in this investigation was obtained from an adult male animal (Struthio camelus) which had been in captivity in Edinburgh Zoo. Its diet consisted of pasture supplemented with whole maize, contrasting with its natural diet which consists of small animals, birds, snakes, lizards and insects, as well as grass, leaves, fruits, berries and seeds. 'A post-mortem revealed a knotted mass of grass filling the proventriculus and gizzard. A varied bacterial flora was isolated from the bone marrow and there was severe congestion of the duodenum and a fatty infiltration of the liver' (Information kindly supplied by Mr. E.C. Appleby).

The sample of flamingo fat came from a fairly young adult bird (Phoenicopterus chilensis) in captivity at Bristol whose diet consisted mainly of vegetable roughage from the bottom of a pond together with worms, slugs and occasionally shrimps. In its natural state the flamingo subsists on the small animals and vegetable matter (algae) which live in the muddy beds of ponds and lakes. This animal's decease was caused by a fracture of the ribs which tore a lung and caused haemorrage. The fats were extracted in the usual way with petroleum ether (b. pt. 40-60°C) and analysed. Detailed results are given in the appendix (page 92).

Unsaturated Acids of Ostrich and Flamingo Fats

Unsaturated C_{16} acids: Both these fats were found to contain hexadecadienoic on the basis of spectroscopic data. Thus the U.V. absorption after isomerisation of a fraction from ostrich fat consisting mainly of C_{16} esters indicated an appreciable $E^{1\%}_{1cm}$ value (273.7) at 234mμ and a much smaller value (15.0) at 268mμ. After allowing for the small quantities of polyethenoid C_{18} acids present, these values were 121.8 and 0.5 respectively. The former value, was taken to indicate the presence of hexadecadienoic acid (12.1%) in the fraction. Bromination of this fraction however gave a white powder m.pt. 176-179°C which was

insoluble in light petroleum and appeared to be hexabromopalmitic acid (Found Br 66.0% calculated for $C_{16}H_{20}O_2Br_6$: 65.7%). The small amounts of hexadecadienoic acids reported in these analyses should thus be regarded as an unidentified mixture of unsaturated C_{16} acids. Clément and Meara (1951) have also reported the presence of hexadecadienoic and hexadecatrienoic acids in rabbit fat solely on the basis of spectroscopic analysis. Hexadecadienoic acid has also been observed in the seed fat of Acacia giraffae (Harrison and Hawke 1952) - in this case its presence is based on the results of an oxidation procedure.

Unsaturated C_{18} acids: As has been noted previously the identity of octadeca di-and-trienoic acids in animal fats with linoleic and linolenic acids has not yet been demonstrated, although the fat of C. porosus did seem to contain polyethenoid C_{18} acids mainly of this structure. In the case of the ostrich fat the structure of these acids is not so certain for the following reasons:-

i) Herb and Riemenschneider (1952) have suggested that replacement of the 7½% potassium hydroxide/glycol solution as the isomerisation reagent by a 21% solution giving larger constants should increase the sensitivity of the procedure for all the polyunsaturated acids except linoleic acid for which the sensitivity is unchanged. Using this method

therefore, if the unsaturated C_{18} acids are mainly linoleic and linolenic acids, a slight increase in the absorption at 268mμ due to linolenic and no change in the value at 234mμ for linoleic acid would be indicated. A suitable fraction from ostrich fat was examined in this way i.e. using a 21% reagent. The results differed from those obtained by the standard procedure as shown in Table 7.

Table 7 - Isomerisation of C_{18} polyethenoid acids

Values are % wt. of fraction.

Glycol Reagent %	Octadeca-trienoic Acid	Octadeca-dienoic Acid	Octadec-enoic Acid	Total
7½	15.2	55.7	27.3	98.2
21	10.6	59.0	34.7	104.3

These discrepancies suggest that the fraction does not consist only of the all cis forms.

ii) Bromination of a fraction rich in C_{18} unsaturated acids (Markley 1947) showed the presence of linoleic acid (22%) and linolenic acid (9%) whilst alkali isomerisation gave values of 46.4 and 10.8% for octadecadi-and-trienoic acids respectively. The large differences in the values for linoleic and octadecadienoic acids is probably significant

and casts further doubt on the homogenity of the octadecadienoic acid.

However, in the case of flamingo fat, bromination of a fraction rich in C_{18} polyethenoid acids indicates the presence of linoleic acid (42.7%) whilst alkali isomerisation gives the value 38.3% for octadecadienoic and 2.2% for octadecatrienoic acid. It has been shown that if the amount of linolenic acid is small and the quantities of linoleic and oleic acids are large, the ether insoluble hexabromostearic acid may not separate from the reaction mixture as may have happened in this case. This result seems to imply then that the octadecadienoic acid is essentially the all cis isomer.

Unsaturated C_{14} acid: An attempt to prepare dihydroxy myristic acid from the fraction richest in tetradecenoic acid gave a very small quantity of a product m.pt. 118-119°C. Unfortunately there was not enough material for further investigation. A dihydroxymyristic acid m.pt. 118-119°C has been obtained from a tetradecenoic acid present in tsuzu oil (Tsujimoto 1928). This acid has been shown to be 4:5 tetradecenoic acid [cf. m.pts. of 9:10 dihydroxy myristic acids of 123°C and 81.5°C - Atherton and Meara 1939].

Further details of acids and derivatives isolated can be obtained by consulting the appendix.

Discussion

The ostrich is reported to be the largest living bird and belongs to the super order Ratitae - a group of birds that shows more primitive characteristics (especially of the palate) than the rest of the family. All the birds in this group are flightless and they are often large with reduced wings and sternum, long legs and curly feathers. This association of failure to fly and primitive condition of the palate has been used to support the contention that these birds have never passed through a flying stage. More probably the ratites represent a population that diverged early from the main stock of flying birds.

The flamingo belongs to a different subclass (Neognathae) and is placed in the same order as the stork and the heron. Notwithstanding these large differences in characteristics, comparison of the fats of these two birds and of other birds show many similarities (Table 8). Some seabird fats which are rather different in composition (Lovern 1938) have not been included.

Table 8 - Component Acids of some Bird Fats (% wt.)

Acid	Grey Goose	Emu	Light Sussex Hen	Ostrich	Flamingo
(Iodine value)	57.1	65.8	78.5	80.4	65.8
Lauric	12.3	-	-	-	-
Myristic	8.2	0.9	1.2	0.9	-
Palmitic	20.3	17.5	24.0	24.8	24.8
Stearic	5.6	10.1	4.1	5.9	7.7
Arachidic	-	0.6	-	0.4	-
Tetradecenoic	0.6	0.9	-	0.9	-
Hexadecenoic	2.5	2.1	6.7	6.1	4.9
Octadecencic	41.6	62.2	42.5	39.8	53.4
Octadecadienoic	6.6	5.2	20.8	17.1	7.1
Octadecatrienoic	-	-	-	3.8	0.2
Unsaturated C_{20-22}	2.3	0.5	0.7	0.3	1.9

The values for the grey goose and the emu are recorded by Hilditch Sime and Maddison (1942) and for the light Sussex hen by Hilditch, Jones and Rhead (1934).

In several respects the figures for grey goose are rather anomalous and as Hilditch, Sime and Maddison (1942) point out this may be due to the presence of coconut oil in the diet, and consequently these figures are neglected

in making any generalisations. Further, the values for the emu do not fit very well into a general scheme, although this bird is related to the ostrich being a member of the Ratitae. The three analyses of the hen, ostrich and flamingo however agree surprisingly well and the following generalisations can be made:-

1) Saturated acids account for 29-32% of the total - a figure which is remarkably constant in the four analyses for the emu, hen, ostrich and flamingo in view of their different I.V.'s.

2) The content of palmitic acid is also constant at about 24-25% (except in the case of the emu) and is slightly below the value of 30±3% (mol.) said to be characteristic of higher land animals (Banks and Hilditch 1931; Hilditch and Longenecker 1937).

3) The lower values for stearic acid parallel those noted for reptiles, amphibians and rodents and are in marked contrast to the greater values of many land animals. The results for the emu are again anomalous.

4) Unsaturated C_{16} acids attain a value (4-7%) which recurs frequently in bird and rodent fats.

5) Unsaturated C_{18} acids are also surprisingly constant and once again with the exception of the emu, comprise about 62% of the total acids of bird fats. Furthermore the unsaturation of these fats seem to be largely controlled by

the relative amounts of the various C_{18} unsaturated acids, an increase in I.V. being accompanied by an increase in polyethenoid acids and a decrease in the oleic acid content. Thus, the low I.V. flamingo fat contains a considerable quantity of oleic acid and the higher I.V. ostrich fat less oleic acid and more polyethenoid acids. The intermediate I.V. hen fat also supports this contention. Winter and Munn (1953) have reported similar observations in the C_{20} acids of seal oils.

6) There are small but definite amounts of C_{20-22} unsaturated acids present in each case, contrasting with the slightly higher values recorded for amphibians and reptiles (cf. Table 6).

A further more general discussion of bird fats is given in the conclusion to Part I of this study.

Analyses of Rabbit, Mouse and Porcupine Fats

Introduction

The detailed study of the component acids of rodent depot fats is almost entirely confined to those of one species - the white rat which is employed extensively in the biological evaluation of vitamins A and D. This circumstance accounts for the comparatively large amount of work which has been carried out on the fats of this animal, particularly concerning the effect of diet on the composition of the depot fat (see Hilditch 1947e). However two analyses of rabbit fat (Shorland 1953: Clément and Meara 1951) and one of guinea pig fat (Baldwin and Longenecker 1944) provide further data for a survey of the fats of rodents. A few investigations of rabbit fat had been completed before these detailed analyses, and although only the I.V. and other constants were recorded, the figures obtained were interesting in that they show a considerable difference in unsaturation between the fats obtained from the wild and tame animals. All the available data are collected in Table 9.

Table 9 - Unsaturation of Wild and Tame Rabbits

Wild Rabbit		Tame Rabbit	
I.V.	Observer	I.V.	Observer
99.8	Amthor and Zink (1897)	72.3	Clement and Meara (1951)
149.6	Shorland (1953)	66.3	" " " "
124	Vickery - as reported by Hilditch 1947f.	67.6	Amthor and Zink (1897)
		76.9	Present work

Mouse and porcupine fats do not appear to have been examined previously.

Origin and Preparation of the Fats

The rabbit fat came from the abdomen of a medium size albino rex female rabbit (Lepus cuniculus) whose diet consisted mainly of bran, dried grass and similar vegetable material (although about 8% of linseed cake was included). The sample of mouse fat came from the abdominal deposits of about 80 animals of both sexes of a strain selected for large size (Mus musculus). The diet consisted of a variant of the Aberdeen Rat Cake (containing 5% white fish meal and 1% cod liver oil). The porcupine fat was procured from the kidney, lungs, heart, spleen and stomach wall of a female crested porcupine (Hystrix cristata) which had been living in Manchester Zoo. The fats were treated and extracted in the usual way. It was found

that some of the fats deteriorated on standing; this was particularly noticeable in the case of the rabbit fat - the original I.V. of the fat on extraction was 89.2. Nevertheless it was felt that an analysis would still give figures of considerable comparative value.

The characteristics of the fats from these animals, together with the analytical results and derivatives isolated are given in the appendix (page101).

Discussion

The animals commonly known as rodents are certainly the most successful of modern mammals - they live in all parts of the world and nearly three thousand species are known, as many as in all the other mammalian orders put together. In spite of the similarities of all animals with gnawing teeth, zoologists consider that the rabbits and hares are not very closely related to the others and are therefore placed in a distinct order, Lagomorphia (Duplicidentata), the order Rodentia being retained for all other 'rodents'. The two orders are placed together in an isolated cohort Glires.

The order Rodentia is further subdivided into three sub-orders one of which (Myomorpha) includes the family Muridae which is comprised of rats and mice and another sub-order (Hystricomorpha) contains the family Hystricidae

which includes the porcupine and the Caviidae which embraces the guinea pig. Thus, it is evident that zoologically, the rabbit must be considered as somewhat different from other 'rodents', being in a different order, and that mice and rats are in a different sub order from the porcupine and guinea pig.

However these zoological differences are not manifested in the fat analyses as reference to Table IO will show.

Table 10 - Component Acids of Rabbit and Rodent Fats (% wt.)

Acid	Rat*		Guinea Pig		Rabbit				Mouse	Porcupine
	male	female	♀ (i)	(ii)	(i)	(ii)	(iii)	(iv)		
(Iodine Value)			-	-	72.3	66.3	149.6	76.9	-	-
Lauric	-	-	-	1.1	0.4	2.4	-	-	-	-
Myristic	1.6	1.8	3.7	5.3	5.5	3.8	1.6	2.6	0.2	5.2
Palmitic	21.6	24.4	22.7	19.4	30.5	29.0	22.1	25.1	26.7	36.3
Stearic	3.6	3.6	7.3	5.7	5.0	4.0	6.4	5.6	2.6	11.7
Arachidic	2.0	0.8	-	-	-	-	0.8	0.4	-	-
Tetradec-enoic	-	0.3	0.4	0.8	-	1.5	0.4	2.2	-	1.5
Hexadec-enoic	4.1	4.8	1.7	2.1	5.4	6.6	4.4	6.0	5.6	3.6
Hexadecad-ienoic	-	-	-	-	0.5	0.3	-	-	-	0.5
Octadec-enoic	51.9	44.3	34.2	36.2	31.9	36.7	12.7	26.5	35.8	27.1
Octadecad-ienoic	13.0	18.6	13.4	18.8	16.3	11.8	7.9	26.0	26.2	13.6
Octadeca-trienoic	-	-	2.0	1.2	3.1	2.0	42.4	5.6	1.9	0.5
C_{20-22} acids	2.2	1.4	9.6	9.4	1.4	1.9	1.3	-	1.0	-

* % mol.

References: Rat (Longenecker 1939)

Guinea Pig (Baldwin and Longenecker 1944)

Rabbit (i) and (ii) (Clément and Meara 1951)

Rabbit (iii) (Shorland 1953)

The other results are from the present work.

The analysis of the guinea pig fat was carried out on two groups of animals which had been fed known diets (containing 28.6% butterfat) on a paired feeding experiment for several weeks before death. The first group had received only a scorbutogenic diet and were in the last stages of scurvy at the time of death. The other group were allowed to consume no more of the diet than their paired fellows in in the first group, but the diet of the second was supplemented with ascorbic acid. No indications of scurvy were present in the latter animals but the first stages of inanation had become apparent. These results for the guinea pig should thus be treated with caution as the diet containing butterfat, and the inanation of the animals might have led to some differences in the fatty acid composition e.g. a decrease in the palmitic acid content (cf. Holmberg 1954).

Nevertheless the following points are worthy of note:-

1) The analysis of the porcupine fat shows a very unusual proportion of saturated acids (53%) and its high content of palmitic acid is also exceptional.

2) Nearly all these animals show a figure for hexadecenoic acid of about 4-7% - a quantity which seems to be characteristic of bird and rodent fats (cf. page 40) and compares with the slightly higher values observed in reptiles and the still higher values of the marine species.

3) The content of palmitic acid is on average slightly below that characteristic of the larger land animals (i.e. 30±3% mol.), although two of the rabbit analyses are within this range, and the porcupine fat is outwith the upper limit of 33%.

4) The contents of stearic acid are parallel to those noted for the birds and the reptiles and are in marked contrast to the 'stearic-rich 'fat of the'higher' animals.

5) In comparing the results for the rabbit fats, the present values seem to be intermediate between those obtained for the wild rabbit by Shorland (1953) and those of the chinchilla rabbit by Clément and Meara (1951). The unsaturated C_{18} acids are present in greater quantities and are more highly unsaturated in the fat of the wild rabbit at the expense of the saturated acids (the contents of the other unsaturated acids being much the same). The unusually high quantity of linolenic acid observed in the fat of the wild rabbit seemed to be due to the diet of pasture grass - the fat of which contains a high quantity of linolenic acid. This suggests that the depot fats of rodents are considerably effected by their diet. It is interesting to note that a horse fed on the same pasture did not show quite such a high quantity of linolenic acid in its fat, although this was greater than when fed on other diets (Shorland 1953). Thus the diet of the rabbit giving the

most saturated fat (the chinchilla rabbit) consisted of rat cake of low fat content (5.6%) whilst the rabbit in this case was fed on a diet containing much more fat (including 8% linseed cake) and gives accordingly a more unsaturated fat with a higher content of linoleic and linolenic acids. The different palmitic acid contents of the rabbit fats also support the contention that the more unsaturated fats contain more ingested unsaturated acids, since it is natural that the proportions of palmitic acid should be reduced when more unsaturated fats containing less palmitic acid are deposited in the depots. These facts probably explain the discrepancies apparent in Table 9 in the fats of wild and tame rabbits - the animal in its natural state consuming exogenous fat of a higher unsaturation. It appears possible then that the results for the chinchilla rabbit are those most representative of the essential fat of the animal and these indeed seem to fit better into the general scheme, in which it is postulated that the fats of rodents are similar to those of birds in being intermediate btween the fats of land and marine animals, but are closer to the land animal type of fat than that of the amphibian and reptile class.

Analysis of Antelope Fat

Introduction

The antelope belongs to the group of animals known as ruminants. These animals possess a digestive system which enables them to convert the cellulose in their food into organic acids which are absorbed in the circulation, presumably forming depot fat. Antelope fat does not appear to have been examined previously, although there have been analyses of some of the related animals including a relatively large number on the more common animals of this group, such as pigs, sheep and cattle (see Table 11).

Origin and Preparation of the Fat

This sample of fat was obtained from the abdomen of a Harnessed antelope (*Tragelophus scriptus*), which had been kept in captivity in Edinburgh Zoo. The animal was reported to have been fed on a diet of maize, green vegetables and carrots, death being caused by congestion of the lungs. The fat was treated and extracted in the usual way giving a pale yellowish brown semi-solid fat S.E. 281.0, I.V. 83.6 and F.F.A. (as oleic) 10.3. The fat had rather an objectionable odour probably due to the relatively high content of free fatty acid.

The analysis was carried out in the usual way, the results and list of acids and derivatives separated being

recorded in the Appendix (page115).

One of the A fractions richest in octadecenoic acid was examined by means of infra red spectroscopy to ascertain the amount of trans acids present in the sample (Knight, Heether, Shreve and Swern 1950). On measuring the absorption at 10.36μ it was found that negligible quantities (if any) of trans acids were present (for significance of these results see discussion below).

Discussion

The antelopes belong to the family Antilocapridae: other families in this group of the Ruminantia include the Cervidae (deer) and the Bovidae (sheep, cattle and goats): closely associated with the ruminants are the Suiformes (pigs and hippopotamii) and the Tylopoda (camels).

It has been suggested that the composition of the depot fats of ruminants is not effected by the dietary fat to any considerable extent (Shorland 1953). Thus the main dietary fatty acid constituent of pasture fed animals - linolenic acid, although present in the fats of non-ruminants (e.g. rabbit and horse) in appreciable quantity, appears only in traces in the depot fats of ruminants. It has been postulated that this can be attributed to modification of the dietary fat in the rumen. Indeed recent work (Johns, Shorland and Weenink 1955) has shown that the rumen functions as a powerful hydrogenating medium and linolenic, linoleic and

oleic acids are readily converted to the mono-ene and the fully saturated stearic acid. It has also been demonstrated that the fats of pasture fed ruminants contain trans acids (Hartman, Shorland and McDonald 1954). It is well known that the hydrogenation of unsaturated fatty acids is accompanied by the formation of trans isomers, and it was therefore suggested by Hartman et al. that this process is responsible for their formation in ruminants. This specimen of antelope fat however does not appear to contain detectable quantities of trans acids. This fact also accords with the general observation that antelope fat seems to be considerably different in the quantitative distribution of the fatty acids from other ruminants and related animals (see Table 11).

Table 11 - Depot Fats of Ruminants and Related Animals (% wt.)

Acid	Deer (i)	Deer (ii)	Sheep	Ox	Camel	Pig	Hippo.	Antelope
Myristic	4.4	5.2	4.8	3.0	6.3	1.3	2.3	2.8
Palmitic	25.1	35.9	25.0	29.2	28.8	28.3	27.1	20.6
Stearic	35.4	29.6	22.2	21.0	27.4	11.9	22.2	3.8
Arachidic	1.5	2.9	0.7	0.4	1.6	-	1.1	0.4
Tetradecenoic	0.5	0.2	0.5	0.6	0.5	-	0.4	0.4
Hexadecenoic	2.8	2.2	1.7	2.7	3.2	2.7	2.2	8.7
Octadecenoic	25.2	17.0	44.2	41.1	26.4	47.5	39.3	40.4
Octadecadienoic	2.6	1.2	-	1.8	1.9	6.0	3.5	19.2
Octadecatrienoic	2.5	1.0	-	-	0.9	-	1.5	3.7
C_{20-22} acids	-	4.8	0.9	0.2	3.0	2.0	0.4	-

References: Deer (i) and camel (Gunstone and Paton 1953a)

Deer (ii) and sheep (Shorland 1953)

Ox (Hilditch and Longenecker 1937)

Pig (Hilditch, Lea and Pedelty 1939)

Hippopotamus (Barker and Hilditch 1950)

Antelope (present work).

In table 11 it will be seen that with the exception of antelope fat, all the results are considerably different from those obtained so far in this study. This is so particularly in the content of stearic acid. Palmitic acid is, in general, within the range of 30±3% (mol.) characteristic of land animals. The sample of antelope fat in addition to its very low content of stearic acid, does not come near this characteristic figure. Furthermore it is evident that the content of hexadecenoic acid is surprisingly constant at about 2-3% - a value a little lower than has been found in the fats of reptiles, birds and rodents. (cf. page 47). Here again, the contents of hexadecenoic acid in the antelope fat does not agree with this observation, and in fact this fat, but for the absence of C_{20-22} unsaturated acids approaches that of a typical reptile. There are a few other cases of animal fats from higher land animals containing similar quantities of stearic and hexadecenoic acids e.g. badger (Gupta, Hilditch and Meara 1950), giant panda and Ceylon bear (Hilditch, Sime and Maddison 1942).

Holmberg (1954) pointed out that in starving animals an increase in the amount of hexadecenoix acid and a decrease in the palmitic acid content was apparent. As this specimen of antelope fat shows an unusually low proportion of palmitic acid and a high content of hexadecenoic acid, it may be that starvation caused the anomalous results (no information

was available from the zoological authorities on this conjecture). Nevertheless it is clear by the absence of trans acids and the very low quantities of stearic acid that the mechanism as proposed by Shorland and co-workers (loc. cit.) for ruminants does not function in this case. This may be because the diet of the antelope in this instance did not contain a fat which is as highly unsaturated as in its natural environment. (i.e. the animal was not pasture fed). However, clarification of these discrepancies awaits further analyses of the fats of animals in this group.

Analysis of Sea Lion Oil

Introduction

The sea lions (Otariidae), walruses (Odobenidae) and seals (Phocidae) have become a large marine order of the carnivores (Pinnipedia). The fats of members of this order have been examined relatively well and in particular extensive analyses have been carried out on the various species of seals. These analyses are noted for the unexpected variations in the compositions of the fats (Burke and Jasperson 1944; Hilditch and Pathak 1947, 1949; Winter and Nunn 1950, 1953; Cardin and Meara 1953). Several less detailed analyses have been carried out (e.g. Ljubarsky 1898; Gansel, 1926; Bauer and Neth 1926; Williams and Makhrov 1935; Dugal 1953).

This analysis is the only one that has been carried out on the oil from a sea lion.

Origin and Preparation of the Oil

The sample of oil was obtained from a male animal (*Otaria gillespii*) aged about 15 months which had been in captivity in Calderpark Zoo (Glasgow) for about 2 months. The fat was extracted in the usual way giving an oil I.V. 173.1, S.E. 288.8, F.F.A. (as oleic) 2.7.

The analysis was carried out by the usual method giving results as shown in the appendix (page 120).

The Unsaturated Acids of Sea Lion Oil

As will be seen on perusal of the results, the unsaturated acids have merely been recorded with their mean unsaturations. No further chemical investigation into the nature of the acids present in the oil was undertaken, since most of the fractions consisted of complex mixtures. The mean unsaturation of the various groups of acids were determined by a simple mathematical method - a variant of the graphical method (see Hilditch 1947d).

However, interesting information was obtained by making use of isomerisation with a 21% solution of potassium hydroxide in ethylene glycol at 180°C for 15 minutes (Herb and Riemenschneider 1952). On measuring the U.V. absorption spectra of the isomerised fractions, the presence of at least pentaethenoid acids was indicated by absorption maxima at 233, 270, 282, 304, 317, 332 and 348 mμ as shown in Figure 2 (cf. Herb, Witnauer, and Riemenschneider 1951).

This is true of fractions which are postulated to contain C_{20} acids (e.g. fraction C11) as well as these containing C_{22} acids (e.g. fraction C17). The occurrence of highly unsaturated C_{20-22} acids of this nature is a characteristic feature of most marine oils.

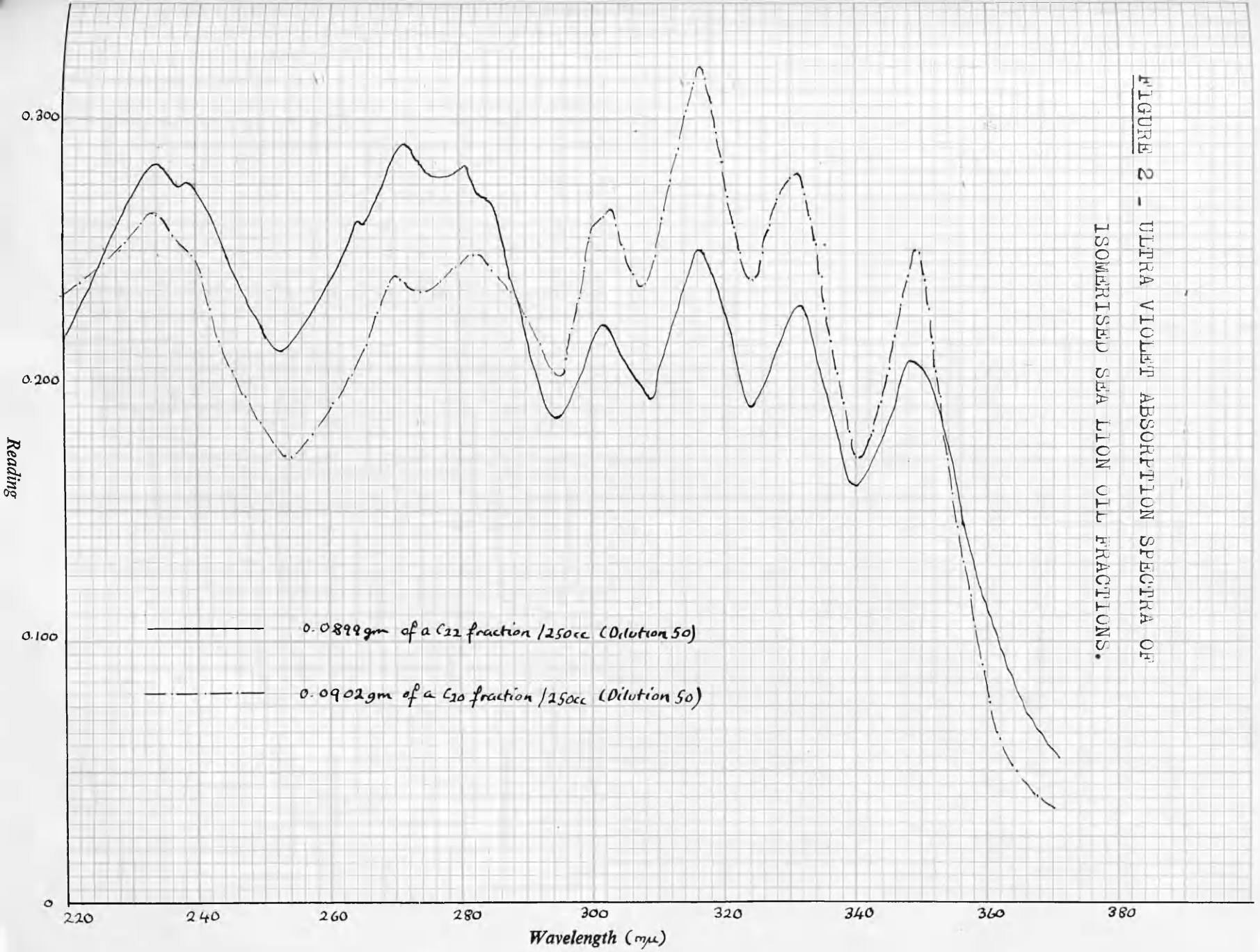


FIGURE 2 - ULTRA VIOLET ABSORPTION SPECTRA OF ISOMERISED SEA LION OIL FRACTIONS.

Measurement of the U.V. absorption after alkali isomerisation of a fraction postulated to contain C_{18} esters only (S.E. 291.1) showed a considerable absorption at 300 and 315mμ. ($E^{1\%}_{1cm}$ of 117.8 and 106.9 respectively) suggesting the presence of tetraethenoid acids (cf. Herb and Riemenschneider 1952). Similar measurement of a fraction consisting of C_{16} esters (S.E. 264.4) showed absorption maxima at 300 and 315mμ having $E^{1\%}_{1cm}$ of 64.3 and 55.5 respectively, also implying that tetraethenoid acids are present. These facts signify the existence of C_{18} and C_{16} tetraene acids in this oil.

A conjugated C_{18} tetraene acid has been found in certain seed oils (parinaric acid) and measurement of the U.V. absorption shows similar maxima to the above (Kaufmann, Baltes, Volbert and Brockhausen 1950). However since the U.V. of the unisomerised acids of the oil showed no appreciable absorption the presence of this acid as such is ruled out, although the isomerised acids may be very similar to it. From Japanese sardine oil Toyama and Tsuchiya (1935) obtained octadeca-4:8:12:15-tetraenoic acid (moroctic acid), which has also been found in bonito oil (Matsuda 1942). On the other hand, it is very doubtful if an acid of this constitution would exhibit tetraene absorption after alkali isomerisation under the standard conditions, since three of the double bonds are separated

by two methylene groups [cf. Farmer 1942; Toyama and Yamahato 1953a]. An interesting C_{18} tetraene acid has been found in the fresh water algae Chlorella pyrenoidosa and it has provisionally been given the structure octadeca-6:9:12:15-tetraenoic acid (Paschke and Wheeler 1954). This acid exhibited U.V. absorption maxima at 234, 268 and 315 mμ and it seems likely therefore that the C_{18} tetraene acid present in this sample of sea lion oil will be similar to this acid, although it must be borne in mind that the observation of the tetraene might possibly be due to the presence of small quantities of a C_{20} tetraene acid which have distilled over early in the ester fractionation. A C_{18} tetraene acid has been found in the depot fat of a frog (Rana tigrina), but no information is available about its structure. (Khalkar, Pholnikar and Bhide 1946).

A similar situation is apparent as regards a hexadecatetraenoic acid - sardine oil contains a hexadeca-4:8:11:14-tetraenoic acid (Tutiya 1940, Toyama and Yamahato 1953b) and the fresh water algae Chlorella pyrenoidosa contains a 4, 7, 10, 13 (all cis) C_{16} tetraenoic acid. Since there is no appreciable U.V. absorption before isomerisation it appears likely that the C_{16} tetraene acid present in sea lion oil is similar to that present in the algae.

These surmises seem all the more likely when it is recalled that the diet of a sea lion consists entirely of fish and that it has been suggested (Paschke and Wheeler 1954) that the fat of fishes follows that of the predominant algae of their environment, and although the algae Chlorella pyrenoidosa is a fresh water type it is possible the C_{16} acids of the marine type will have a similar structure.

Discussion

When the range of fatty acid distribution in the various specimens of blubber fat of seals is compared with that obtained in this case (see Table 12) it is evident that there is a broad similarity between all the seal blubber fats. Somewhat larger amounts of C_{16} unsaturated acids are present in the blubber fats of the common seal and crabeater seal, but since a large variation in the C_{16} unsaturated acids has been found in various animals of the same species (elephant seal) it is doubtful whether these greater amounts of C_{16} acids can be considered as a specific feature of these species.

Table 12 - Composition of Blubber Fats of Various Seals
(% mol.)

Acid	Elephant Seal	Grey Seal	Leopard Seal
Lauric	0-0.4	-	-
Myristic	1.1-6.3	4.6	4.9
Palmitic	8.2-14.0	11.5	8.1
Stearic	2.1-4.6	2.0	1.6
Arachidic	0-0.9	-	0.2
Unsaturated C_{14}	0.6-2.3 (-2.0H)	2.1 (-2.0H)	2.6 (-2.0H)
Unsaturated C_{16}	8.2-17.8 (-2.0 to -22H)	17.2 (-2.2H)	14.1 (-2.1H)
Unsaturated C_{18}	32.9-44.4 (-2.1 to -2.7H)	30.9 (-2.7H)	42.2 (-2.2H)
Unsaturated C_{20}	11.7-25.3 (-2.8 to -6.7H)	15.2 (-5.7H)	15.8 (-4.6H)
Unsaturated C_{22}	5.9-13.2 (-4.9 to 10.8H)	15.5 (-10.6H)	10.5 (-9.4H)
Unsaturated C_{24}	0 - 3.1 (-2.0 to -3.9H)	1.0 (-11.0H)	-

With the exception of the present results, these values are as tabulated by Winter and Nunn (1953).

Newfound-land seal	Common seal	Crabeater seal	Sea lion
trace	-	-	-
6.6	2.7-2.9	5.7	4.9
11.7	11.1-11.4	10.9	13.8
1.3	2.4-4.3	1.6	2.7
0.5	0.3	-	-
2.2 (-2.0H)	2.7-3.6 (-2.0H)	3.9 (-2.0H)	2.5 (-2.0H)
11.6 (-2.14)	22.7-27.8 (-2.1H)	21.5 (-2.3H)	13.4 (-2.4H)
39.5 (-2.4H)	33.2-33.7 (-2.4 to -2.7H)	30.0 (-3.2H)	30.7 (-2.6H)
16.0 (-5.6H)	12.4-13.6 (-5.9 to -7.2H)	17.5 (-7.9H)	21.6 (-7.5H)
9.0 (-9.3H)	10.3-12.2 (-11.0H)	8.9 (-10.8H)	10.4 (-10.7H)
1.6 (-10.9H)	-	-	-

It can be seen that all the analyses of seal oils show a similarity in their small quantities of saturated acids, coupled with the large quantities of highly unsaturated C_{20-22} acids - a characteristic feature of all marine fats (cf. Hilditch 1947g). Analyses of cammivores has so far shown that their body fat composition is governed to a large extent by their diet (Gunstone 1955b). That this is not entirely the case for seals is shown by the work of Winter and Nunn (1950) who demonstrated for example that in spite of the large differences in composition between seals of the same species that the blubber oil of a Macquarie Island mature male elephant seal did not differ greatly from the blubber oil of a Heard Island mature male leopard seal, although this specimen is recognised to feed on a more elaborate diet than the elephant seals. Cardin and Meara (1953) in a study of the lipids of the grey Atlantic seal concluded that from the time the seals receive a natural marine diet (after suckling), the content of saturated acids, in particular of palmitic acid, decreases at the expense of C_{20-22} unsaturated acids, making the I.V. a function of the age of the animal. This sample of sea lion oil is interesting in the fact that it is the most unsaturated so far examined, and while it is difficult to make any generalisations in the absence of data on other

animals of this species, it should be noted that the saturated acids form about 21% of the total acids, in comparison to an average value of about 18% for other seals. This difference is counterbalanced by an increase in the unsaturation of the C_{20} acids, a fact which is further indicated below. This does not altogether accord with Cardin and Meara's postulate that the saturated acids decrease with increase in I.V. On the other hand, Winter and Nunn (1953) observed that the lower I.V. fats have a relatively low content of saturated acids (15%) compared with the more highly unsaturated fats which contain up to 22% of saturated acids. These authors also discussed the saturation and desaturation processes occurring in seal oil and they note that major variations are to be found mainly in the group of acids having a chain length of C_{20} or more and the unsaturation value of the C_{18} acid groups remain substantially constant. (The 'unsaturation value' is defined as the product of the molar percentages of the fatty acid group and its mean unsaturation divided by 200 - thus representing the number of double bonds contributed to one average molecule of fat by each group of fatty acids). Based on their 16 analyses of elephant seal oil Winter and Nunn (1953) have compiled limits for the unsaturation values of the various acids as they occur in the oil. These limits and the unsaturation values for the present sample of sea lion oil are shown in Table 13.

Table 13 - Unsaturation Values of Seal Oils

Chain Length	Unsaturation Value Limits	Unsatn. Values of Sea lion oil
C_{14}	0.01 - 0.02	0.02
C_{16}	0.08 - 0.19	0.16
C_{18}	0.38 - 0.48	0.40
C_{20}	0.26 - 0.62	0.81
C_{22}	0.30 - 0.63	0.55
C_{24}	0.01 - 0.06	-

It is noteworthy that the unsaturation values of the sea lion oil fall within the prescribed limits, with the exception of the C_{20} acids which seem to be more unsaturated than any so far encountered in this group of animals. Unfortunately, no further investigation of these acids was carried out as the present methods of investigating polyethenoid acids are not very satisfactory (cf. Part II).

General Conclusions and Discussion

I As has been already indicated (page 2) this study of animal fats is a continuation of work commenced in this laboratory in 1950, and it is convenient at this stage to pass comment on the analytical procedures as they have been evolved in these years.

a) It is an important aspect of this work to provide an efficient means of storing the fats, as it is not always convenient to carry out analyses of the fats as they become available, since it is necessary to concentrate on work of a parallel nature to improve these analyses (e.g. Part II). In practice, the fats were extracted immediately they were available and then stored in a sealed container in a refrigerator at -4°C until ready for analysis. In some cases a period of two years had passed before an examination was begun, and it was found that this means of storage was effective in preventing serious deterioration except in the case of some of the more unsaturated fats. The degree of deterioration was measured by noting the drop in the I.V. of the fat from that determined on the freshly extracted fat to that just before the analysis was commenced. It was found that the degree of deterioration was very small in the case of fats of I.V. 80 or less but in some of the more highly unsaturated fats it was quite

marked. Thus, a sample of monitor fat of I.V. 108.0 on extraction showed an I.V. of 81.0 after storage and accordingly no attempt at an analysis was made. The specimen of rabbit fat also appeared to have deteriorated a little; nevertheless an analysis was carried out as usual and it was found, as in other similar cases that any products of deterioration (probably acidic fission products) appeared in the first few fractions of C Fraction resulting in a very low S.E. (e.g. S.E. of Rabbit C1 was 199.4). However, assumptions can be made regarding the compositions of these fractions using the I.V. and S.E. values of the later ester fractions which still seem to give results of fair accuracy.

b) In the procedure of fractional crystallisation a scheme was adopted which gave good separations of the fractions according to their I.V.'s. Thus by recrystallisation at -60°C and then at -20°C from methyl alcohol, a fair separation into polyethenoid, monoethenoid and saturated fractions was achieved. However in some fats in which there was only a small proportion of polyethenoid acids, this procedure while giving excellent separations led to very small C fractions. (e.g. Flamingo: 8.9gm.). The resulting distillation of the methyl esters was thus not so satisfactory and fractions of about 1gm. had to be

collected. Nevertheless using a modification of the macro method for determination of S.E.'s fairly consistent results were achieved. It will thus be evident that in these cases where the polyethenoid content is low (generally low I.V. fats) a compromise must be achieved between an efficient separation of the polyethenoid fraction and the size of the fraction (assuming a limited quantity of fat is available). This suggests that a better analysis would be obtained in these cases by carrying out the first crystallisation at higher temperatures e.g. -40°C instead of -60°C (cf. Brown and Kolb 1955).

c) It is apparent that in the analyses which indicate small quantities of unsaturated C_{20} acids being present, the evidence for these is derived from the S.E. of the residual fractions coupled with a determination of the unsaponifiable material. As an accurate determination of the S.E. of the highly coloured residue is rather difficult and together with the rather arbitrary extraction of the unsaponifiable matter, it seems reasonable to express doubts about the presence of these acids.

Other acids about which there is a certain uncertainty are the C_{16} polyethenoid acids. Thus, in the ostrich analysis, spectral evidence indicated the existence of a diethenoid acid, whilst bromination of the relevant fraction

implied the presence of a triethenoid acid. This might be due to the occurrence of an acid which has three double bonds, only two of which are conjugable. However, further clarification of this and related problems awaits the development of methods of examining the more unsaturated acids (see Part II).

One method of examining unsaturated acids which has become an invaluable tool in recent years is that based on measurement of the U.V. absorption after alkali isomerisation (see Herb 1955). Further standardisation of the methods using a 21% solution of potassium hydroxide in ethylene glycol (cf. analysis of seal oil) should throw some light on the structure of the C_{20-22} unsaturated acids characteristic of fish oils. It was noted however in this method that there appeared to be some difficulties in measuring the U.V. absorption because of the deposition of silica caused by the action of the strong alkaline solution on glass.

II. In 1936, Hilditch and Lovern remarked that the lower forms of life such as fishes deposit fats which are exceedingly complex in their structure, containing as well as C_{14}, C_{16} and C_{18} saturated acids a wide range of unsaturated acids of the C_{14}, C_{16}, C_{18}, C_{20} and C_{22} series. This compares with the fats of the more highly evolved land animals which generally contain as major components only

three acids viz. palmitic, stearic and oleic acids. As more analyses became available it was apparent that there was a class of animals in which the fatty acids were intermediate in character between the two groups. This class of animals also occupied an intermediate position in the evolutionary pattern i.e. the amphibians, reptiles and birds. The analyses completed in respect of this work agree with this contention and comparison with the general scheme of evolution (cf. Young 1950) does indeed show a progressive simplification as the animals become more developed (see Table 14). It will be seen from the table that the absence of C_{20-22} acids and the relatively small proportions of C_{16} unsaturated acids, coupled with increased quantities of palmitic and stearic acids appear to be characteristic of the 'higher' animals, the amphibians, reptiles, rodents and birds holding an intermediate position as regards the proportions of these acids.

Table 14 - Component Fatty Acids of Animal Depot Fats (% wt.)

Class	Animal	Saturated Acids			Unsaturated Acids			
		C_{14}	C_{16}	C_{18}	C_{16}	C_{18}	C_{20}	C_{22}
Fish	Freshwater	3-5	13-19	0-4	0.20	38-45	13-15	0-8
	Marine	3-8	11-19	0-4	5-15	18-30	18-30	8-27
Amphibian	Frog	4	11	3	15	52	15	
Reptile	Tortoise	1	14	4	9	65	7	
	Python	1	20	11	4	58	4	
	Crocodile	4	24	3	15	40	7	4
Bird	Ostrich	1	25	6	7	61	-	
	Flamingo	-	25	8	5	61	2	
Rodent	Mouse	0.2	27	3	6	64	1	
	Rabbit	3	25	6	6	58	-	
	Porcupine	5	36	12	4	41	-	
Ruminant	Antelope	3	21	4	9	63	-	
	Deer	4	25	35	3	30	-	
	Sheep	5	25	22	2	44	-	

Evolution ↓

Increasing simplicity of fatty acids ↓

If the references to these analyses have not been already indicated they will be found in Hilditch (1947). Some animal fats do not fit closely into this scheme e.g. it was found (Gupta, Hilditch and Meara 1950) that badger fat contained almost 15% of unsaturated C_{20} acids and of the

fats analysed in this series, the antelope seems rather anomalous in its low content of saturated acids and high content of C_{16} and C_{18} unsaturated acids.

It should be noted at this point, that with the exception of the dolphin family, whose fats contain considerable quantities of iso-valeric acid, animal fats do not seem to contain as major components such characteristic acids as those of the vegetable fats e.g. hydroxy, epoxy and acetylenic acids as found in certain botanical species (cf. Part III), although branched chain and uneven numbered fatty acids have been found as minor components of some animal fats (Shorland 1952, 1953; cf. Hilditch 1952).

The increasing simplicity in the composition of fats as one proceeds from the 'lower' to the 'higher' forms of life has been discussed by Shorland (1952, 1953) who suggests that this gradation is mostly due to the fortuitous influence of diet. That the diet influences the depot fat of animals has been shown by many observers. Thus, rats fed on corn oil deposit fats containing similar proportions of fatty acids to those present in the diet (Longenecker 1939a). This however cannot be said to be true of all animals or even of the same animals fed on different diets e.g. it has also been shown that rats fed on coconut oil deposit a fat containing considerably less of the lower saturated acids than are characteristic of coconut oil.

(Longenecker 1939b). In this case it must be assumed that the lower saturated acids are preferentially metabolised by the animal. The study of the formation of fats in the animal is an extremely complicated one and it would not be suitable to discuss the present theories of metabolism at this stage (for a Review, see Shorland 1955).

However, it may be said that it has been generally recognised that in the course of evolution there appears to be discernable changes in the nature of the fat metabolism of animals and Shorland (1953) has attempted to classify three types of animal:-

a) species that deposit dietary fat only

b) species that deposit dietary fat and endogenous fat

c) species that deposit endogenous fat only.

It has been emphasised by Shorland that these subdivisions are not rigid but represent an attempt to use a biological rather than an empirical basis for calculation. In the first category he names fish, in the second amphibians, reptiles and non-ruminants (which use carbohydrate and protein to produce endogenous fat); in the third category are found the fats of the ruminants which have been shown (Shorland 1953, Thomas, Culbertson and Beard 1934) to have a fat composition not substantially affected by dietary fat. Nevertheless it has been pointed out by Hilditch (1952) that many animal fats at first sight do not appear to fall smoothly into the above classification. The analyses

completed in respect of this work furnish an interesting addition to the available data and provide further comment on the above classification:

a) Seals have been shown to deposit a marine type of fat, but as has been already mentioned (page 62) there is considerable variation within the different seal types. This variation is not entirely accounted for by variation in the diet and is probably due (Cardin and Meara 1953) to the different metabolic functions which predominate as the animal grows (cf. Callow 1935). Thus it is evident that seals, although depositing a marine fat, do not belong to a species storing dietary fat only.

b) The analyses of crocodile fat provide a good illustration of the effect of diet on fat composition. It can be seen (Table 6) that the fat of the wild animal is much nearer to the marine type as exemplified by the larger quantities of C_{16} and C_{20-22} unsaturated acids. However both types of fat are similar in that they contain parallel quantities of the saturated acids in contrast to the smaller quantities of these acids present in the marine species. An analysis of another animal which although not a true amphibian has a similar environment to that of the crocodile, has been carried out. This analysis of hippotamus fat (Barker and Hilditch 1950) showed, in contrast to the crocodile fat,

a very high content of stearic acid (22%) and only small quantities of C_{16} and C_{20-22} unsaturated acids (see Table 11). The hippopotamus feeds on the lush vegetation abounding at the edges of African rivers and Lovern (1936) has observed that the fat derived from flora of this kind contains substantial amounts of unsaturated C_{16} and C_{20} acids (e.g. about 25 and 12% respectively). It is thus possible that the exogenous fat of both animals i.e. hippopotamus and crocodile, will be similar in constitution, in contrast to the different types of fat laid down in the tissues. These results suggests that the fat of the hippopotamus is derived by a different mechanism (perhaps from carbohydrates and protein in the diet), and they further show the striking influence of species on fat composition in that the hippopotamii are related to the pigs being of the same sub order Suiformes. It is well established that the pig belongs to that group of animals depositing a "stearic-rich" type of fat (cf. Hilditch 1947h).

The analyses of the bird and rodent fats also conform to this intermediate group and on consulting Table 14 the decreased content of C_{16} unsaturated and especially of the C_{20-22} unsaturated acids is noticeable in proceeding in the order fishes ⟶ amphibia ⟶ reptiles ⟶ birds ⟶ rodents, the order suggested by zoologists for the evolutionary process. A further point may be made at this stage regarding the fat

composition of the birds. The two samples analysed in this study (i.e. ostrich and flamingo) are very similar in spite of their different diets. The flamingo is reported to feed mainly on the blue green algae situated at the bottom of ponds and as has already been mentioned (page 59) these algae are characterised by their content of highly unsaturated C_{16} and C_{18} acids, which are not evident in the depot fat. There is a very great variety in the feeding of birds and it would be very interesting and would provide interesting comment on Shorland's postulate, to obtain further analyses from other species of birds to ascertain if this comparative consistency of composition is maintained. Thus, some birds (e.g. thrushes) can eat flesh such as worms which are reported to contain lipids with unusually high quantities of C_{20-22} unsaturated acids (Lovern 1940) whereas other birds (e.g. finches) subsist almost entirely on vegetable matter such as seeds and berries.

The results obtained for the 'rodents' - rabbit and mouse confirm the intermediate characteristics of these fats although the diet seems to have a greater influence on the body fat than in other types of animal. The result for the porcupine is rather unusual in its high content of saturated acids and may be due to a species differentiation in much the same way as the turtle (Chelone mydas) exhibits unusual quantities of the lower saturated acids in its fat (Giral and Giral 1948, Green and Hilditch 1938).

It may be concluded then, that the fats of amphibians reptiles and birds are only effected to a limited extent by their diet and depend mostly on the metabolism characteristic of their species to produce depot fat. On the other hand available evidence points to the important influence of diet on the depot fats of rodents, although this does not explain the anomalous results obtained for the porcupine fat.

c) The last group postulated by Shorland, consisting of these species depositing endogenous fat only is typified by the ruminants in which it is assumed that the organism loses the power to deposit fat from the diet. The ruminant whose fat was examined in this study (antelope) has a fat which appears to be different from any other fat in this group (see Table 11) With the exception of the absence of C_{20-22} unsaturated acids, the fat appears to be more typical of a reptile. The absence of <u>trans</u> acids also confirms the divergence of this analysis from that typified as being characteristic of ruminants by Shorland. In fact, hippopotamus fat with its high content of stearic acid and low content of polyethenoid C_{18} acids (in contrast to its diet), seems to be much more of a typical ruminant fat, and indeed belongs to a related family (Suiformes) The present indications are that there is a group of animals who deposit mainly endogenous fat, although this does not seem to be characteristic of ruminants. However, further investigations on the fats of this type of animal must be made before any definite conclusions can be drawn.

Summing up, it would probably be true to say that in the course of evolution there appears to be gradual changes in the fat metabolism of animals, and that these changes are superimposed on the ability of the animal to deposit fat from the diet, thus making it a very difficult task to discern the ultimate pattern, and that any attempt to make a classification of animal fats at this stage of knowledge (cf. Shorland 1953) seems rather premature.

It will be readily understood that these problems are of considerable importance and that the solution to them awaits the completion of analyses from animals of a wide diversity of species. Experience in this work has shown that the most valuable results will be achieved from those animals in which an adequate degree of control as regards diet has been maintained. Many of the results obtained on animals would have been of much greater value if more information had been available regarding their diet and state of health. Ideally, the best results will be obtained on healthy animals which have lived in their natural environment, a detailed assessment, however, of their diet being available - a requirement which will obviously be impossible to achieve in the case of many animals. Another point on which relatively little information is available is as regards the cause of seasonal variations in fat composition - thus a sample of fat extracted from the turtle Chelone mydas in

winter was substantially different from that obtained from the summer fat (Giral and Giral 1948). In each case the content of fat in the tissues differed considerably and it is probable that some factor such as hibernation or perhaps seasonal variation of diet may account for these discrepancies.

The foregoing will demonstrate the need for further adequately controlled analyses in this field before an effective degree of knowledge can be collected.

III. About 40 analyses have now been completed on various species (excluding marine types) of animals and a perusal of them shows a distinct variation of composition with iodine value. With a few exceptions the 'higher' land animals have a low I.V. and the intermediate type have an I.V. between 65 and 90. The resulting difference in composition can thus to a certain extent be reflected by the I.V. as well as by the species of the animal. Surveying the whole field and taking each acid group in turn the following points are apparent:-

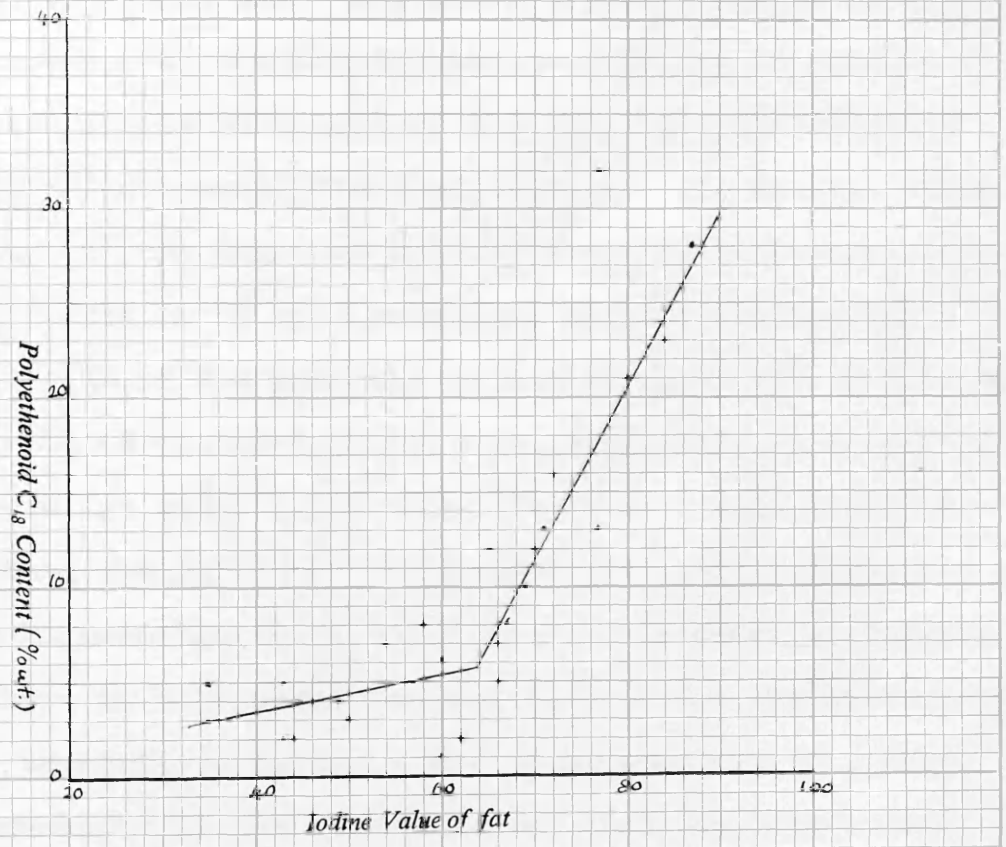
i) C_{12} and C_{14} acids: In general, the contents of these acids are small and there does not seem to be any correlation between quantities of these acids and I.V. (or species). The unsaturated acids are negligible, but myristic acid with a few exceptions, is present in small but definite amounts in most fats and varies from about 1-6% - a good average value being about 3%.

ii) C_{16} acids: With a few exceptions (e.g. antelope fat) the content of palmitic acid remains fairly constant within the region 24-30% - a good average value being about 27% (wt.) although it should be pointed out that this generalisation does not seem to hold for the amphibians and some of the reptiles where the content tends to be much lower. This suggested average figure of 27% (wt.) [i.e. 28-29% (mol.)] compares with the slightly higher range of 30±3%(mol.) put forward by Hilditch and co-workers [Banks and Hilditch 1931; Hilditch and Longenecker 1937] for the 'higher' land animals. The unsaturated C_{16} acids, which appear to be largely palmitoleic acid, although present in small amounts show a definite tendency to decrease with fall in I.V. Thus, fats of the 'higher' land animals with low I.V. generally contain less palmitoleic acid than the 'lower' animals with higher I.V. fats. By plotting a graph of I.V. against unsaturated C_{16} acid content (the polyethenoid C_{16} content is very small or non-existent) an approximate straight line relationship between I.V. and the acid content is evident, and this can be given by 'C_{16}' = (I.V. x .075)%.

iii) C_{18} acids: As these acids generally comprise over 50% of the fat it will be clear that a considerable amount of the saturation and desaturation processes taking place in natural fats will occur in these acids. It has been remarked (Gunstone 1955a) that in herbivorous animal fats as the

iodine value rises, the content of stearic acid falls and that of oleic acid rises. In the higher range of iodine values substantial amounts of octadecadi- and-trienoic acids begin to appear. This statement also seems to be broadly true of all animal fats which have an I.V. of about 90 or less. In the higher I.V. fats the appearance of C_{20-22} unsaturated acids complicates this pattern. Further consideration of the C_{18} unsaturated acids brings to light another interesting fact. It is noticeable that on surveying all the fats that have been analysed, in relation to their I.V., that fats of I.V. 35 to about 65 show fairly small quantities of polyunsaturated C_{18} acids (0-6%), the content of oleic acid being roughly correlated to the increase in I.V. However at about I.V. 60-65, the content of polyunsaturated acids increases and the content of oleic acid although variable tends to fall. This is shown in Figure 3 where the I.V. is plotted against the polyunsaturated acid content (i.e. octadecadi-and trienoic acid). The value of 60-65 for the I.V. at which this change occurs seems rather significant, and a plausible explanation would be interesting. The S.E. of an animal fat is to be found very often in the region of 280; thus considering one typical glyceride molecule containing two double bonds [e.g. $G(SU_2)$] its I.V. will be approximately $127 \times 4/_{3\times280} \sim 61$ Further, since most animal fats generally contain about

FIGURE 3 - VARIATION OF C_{18} POLYETHENOID ACID CONTENT WITH I.V. OF FAT.



30% of C_{16} acids (mostly palmitic) and between 35-65% of C_{18} acids, according to the even distribution rule (Hilditch 1949) a typical glyceride molecule of an animal fat will contain one residue of C_{16} acid (i.e. palmitic acid) and two of C_{18} acids. The above reasoning seems to suggest that in the desaturation processes occurring in fats, a stage is reached when most of the glyceride molecules in the fat will be palmito-diolein i.e. at I.V. 60-65. When this happens any increase in I.V. seems to be brought about by the formation of a polyethenoid residue and not by desaturation of the palmitic acid residue. This supposition can be stated in the opposite way and provides a theory of fat formation which might be worth further consideration:-

Since the I.V.'s of the dietary fats of most animals are rather higher than their depot fats, this seems to suggest that there is some mechanism responsible for the hydrogenation of the unsaturated acids in the exogenous fat, this being most developed in the higher animals such as 'ruminants'. The degree of hydrogenation of this exogenous fat superimposed on the endogenous fat, produced synthetically from carbohydrate and other smaller units, gives the fat characteristic of the species. Thus the fats of reptiles, birds and especially rodents do not appear to be able to hydrogenate their dietary fat to any considerable extent, and these acids coupled with a certain amount of acids formed by

interconversion (see Shorland 1955) gives the fats of I.V. 60-90 characteristic of these groups of animals. The more developed animals (or animals with fats of I.V. 65 or less) show more tendency to hydrogenate their dietary fatty acids. In animals with fats of I.V. 65-90, the hydrogenation occurs mostly in the polyunsaturated acids, thus giving fairly small quantities of stearic acid. At I.V. 65 the fat will consist mostly of glyceride molecules containing one saturated and two monounsaturated residues. Further hydrogenation can then only be carried out on the monounsaturated (C_{18}) acids, thus giving an increase in the stearic acid content (cf. Gunstone 1955a; Hilditch 1947i). It is emphasised that this process will probably be only one of several complicated reactions, and in spite of some fats which do not at first sight agree with this scheme, it offers a plausible explanation of most of the observed results.

In spite of this complicated pattern for the C_{18} unsaturated acids, on plotting a graph of I.V. against unsaturated C_{18} content an approximate straight line relation was observed, given by the expression 'C_{18}' = (35 + I.V.) x 0.54. On plotting the I.V. against stearic acid content, because of the phenomenon of selective hydrogenation about I.V. 60-65, a simple straight line correlation was not apparent. However, by considering fats of I.V. greater than 63 in a separate category, two expressions were evolved:-

For fats of I.V. greater than 63, the stearic acid could be given by the expression $C_{18}^{0} = 15 - I.V. \times 0.125$ and for fats of I.V. less than 63, by the expression $C_{18}^{0} = 45 - I.V. \times 0.55$.

iv) C_{20-22} acids: Arachidic acid although present in some fats is not there in any substantial amount, and its presence does not seem to depend on I.V. - a similar observation can be made regarding behinic acid which occurs only rarely. The C_{20-22} unsaturated acids are nearly always present in small amounts in the fats of intermediate I.V. and although arachidonic acid has been described as an essential acid (cf. Turpeinen 1938) there have been a few analysis in which C_{20-22} unsaturated acids have not been detected. As the animal becomes more marine in character (e.g. an amphibian) then the content of these acids generally increase. However in the fats under consideration in this case (i.e. I.V. less than 90), the presence of C_{20-22} unsaturated acids can be neglected.

Using the simple relationships derived above (i.e. $C_{14}^{0} = 3\%$; $C_{16}^{0} = 27\%$; 'C_{16}' = I.V. x .075; 'C_{18}' = (35 + I.V.) x 0.54; $C_{18}^{0} = 15 - I.V. \times 0.125$ (if $I.V. > 63$); $C_{18}^{0} = 45 - I.V. \times 0.55$ (if $I.V. < 63$), it thus seems possible to predict the approximate compositions of animal fats using their I.V.'s only (if these are less than 90). In this way the composition of the animal fats examined in this study were computed using these expressions, and after correcting to 100%, were compared with the observed results as in Table 15.

Table 15 - Approximate Calculation of Fat Content (% wt.) from I.V.

Acid	Porcupine		Flamingo		Rabbit	
	Actual	Calculated.	A.	C.	A.	C.
(Iodine Value)	50.3	-	65.8	-	76.9	-
Myristic	5	3	-	3	3	3
Palmitic	36	28	25	28	25	27
Stearic	12	18	8	7	6	5
Unsaturated C_{16}	6*	4	5	5	8	6
Unsaturated C_{18}	41	47	60	57	58	59
Unsaturated C_{20}	-	-	2	-	-	-

* Includes unsaturated C_{14} acids

Perusal of this table shows that reasonable agreement prevails except in the case of the saturated acids of porcupine. However, the validity of these conjectures await the further publication of analyses on animal fats.

Ostrich		Crocodile		Antelope		Mouse	
A.	C.	A.	C.	A.	C.	A.	C.
80.4	-	80.5	-	83.6	-	86.8	-
1	3	3	3	3	3	-	3
25	26	27	26	21	26	27	25
6	5	5	5	4	4	3	4
7	6	8*	6	9	6	6	7
61	60	53	60	63	61	63	61
-	-	4	-	-	-	1	-

APPENDIX TO PART I

Complete experimental details of all the animal fat analyses are given in this section.

A. Analysis of Crocodile Fat (See page 23)

Table 16 - Characteristics of Crocodile Fat

	Fat			Mixed Acids	
	I.V.	S.E.	F.F.A. (as oleic)	I.V.	S.E.
C. porosus	80.5	278.9	3.4	83.9	267.4
C. niloticus	93.3	283.2	0.4	96.8	271.5

Table 17 - Fractional Crystallisation of C. porosus Fat.
(corrected weights).

Mixed Acids 206.6gm. I.V. 83.9

10 x MeOH -40°C

Insoluble 126.6gm. I.V. 44.8

Soluble 80.0gm. I.V. 143.5 (Fraction C)

10 x MeOH -20°C

Insoluble 68.5gm. I.V. 10.3 (Fraction A)

Soluble 58.1gm. I.V. 85.8 (Fraction B)

i.e.

Fraction	68.5^{A}	58.1^{B}	80.0^{C}
Weight (gm.)			
% wt. of total	33.2	28.1	38.7
I.V.	10.3	85.8	143.5

Details of the ester distillations and the final calculations of the results for C. porosus fat are given in Tables 1-4 (pages 10 - 22).

In addition to the unsaturated acids discussed on page 24 the following saturated acids were identified in C. porosus fat.

Palmitic acid m.pt. 60-62°C.

Stearic acid m.pt. 66-67°C.

Mixed m.pts. with authentic samples gave no depression.

Table 18 - Fractional Crystallisation of C. niloticus fat

(corrected weights)

Mixed acids 202.6gm. I.V. 96.8

10 x MeOH -45°C

Insoluble 100.2gm. I.V. 39.6

Soluble 102.4gm. I.V. 153.1 (Fraction C)

10 x MeOH -20°C

Insoluble 62.3 I.V. 9.4 (Fraction A)

Soluble 37.9gm. I.U. 89.5 (Fraction B)

i.e.

Fraction	A	B	C
Weight (gm.)	62.3	37.9	102.4
% wt. of total	30.8	18.7	50.5
I.V.	9.4	89.5	153.1

Table 19 - Distillation of A esters from C. niloticus fat

No.	weight gm.	I.V.	S.E.	C_{14}°	C_{16}°	C_{18}°	C_{20}°	C_{16}^{1}	'C_{18}'	N.S.
1	2.52	2.4	254.3	1.37	1.09	-	-	0.06	-	-
2	2.85	3.2	270.1	-	2.75	-	-	0.10	-	-
3	3.22	2.0	270.8	-	3.15	-	-	0.07	-	-
4	2.87	1.6	270.8	-	2.82	-	-	0.05	-	-
5	2.86	1.7	271.5	-	2.81	-	-	0.05	-	-
6	3.24	1.5	270.9	-	3.19	-	-	0.05	-	-
7	3.32	1.1	270.3	-	3.28	-	-	0.04	-	-
8	3.36	0.7	270.4	-	3.34	-	-	0.02	-	-
9	3.35	0	271.0	-	3.35	-	-	-	-	-
10	2.63	0.5	271.2	-	2.62	-	-	0.01	-	-
11	2.85	0.3	270.5	-	2.84	-	-	0.01	-	-
12	3.41	0.7	271.5	-	3.38	-	-	0.03	-	-
13	3.06	4.8	275.0	-	2.51	0.39	-	-	0.16	-
14	3.13	29.7	290.0	-	0.82	1.27	-	-	1.04	-
15	2.59	40.2	295.6	-	0.16	1.27	-	-	1.16	-
16	3.21	28.2	297.1	-	0.01	2.19	-	-	1.01	-
17	4.22	27.0	313.0	-	-	0.67	2.27	-	1.27	0.01
Totals	52.69			1.37	38.12	5.79	2.27	0.49	4.64	0.01
% Esters				2.60	72.34	10.99	4.31	0.93	8.81	0.02
% Acids				2.58	72.25	11.03	4.35	0.93	8.84	0.02
% Fraction (30.8)				0.79	22.25	3.40	1.34	0.29	2.72*	0.01

* 2.60 C_{18}^{1}; 0.12 C_{18}^{11}. Fraction A15 when isomerised at 180°/60min. had $E_{1cm}^{1\%}$ at 234mμ of 18.5. U.V. absorption at other wavelengths, and when unisomerised was negligible.

Table 20 - Distillation of B esters from C. niloticus fat

No.	weight gm.	I.V.	S.E.	C_{14}°	C_{16}°	C_{14}^{1}	C_{16}^{1}	C_{18}^{1}	C_{18}^{11}	C_{20}	N.S.
1	2.51	7.5	244.4	2.22	0.10	0.08	0.11	-	-	-	-
2	2.87	56.5	263.5	0.07	1.15	0.47	1.18	-	-	-	-
3	3.30	59.5	276.4	-	1.17	-	1.21	0.88	0.04	-	-
4	3.21	83.3	293.0	-	0.23	-	0.24	2.62	0.12	-	-
5	3.23	89.3	295.8	-	-	-	-	3.09	0.14	-	-
6	2.87	90.0	295.0	-	-	-	-	2.73	0.14	-	-
7	3.04	89.3	296.1	-	-	-	-	2.89	0.15	-	-
8	2.81	90.4	294.9	-	-	-	-	2.66	0.15	-	-
9	2.74	90.7	295.1	-	-	-	-	2.58	0.16	-	-
10	2.93	89.7	296.0	-	-	-	-	2.79	0.14	-	-
11	2.57	89.1	294.9	-	-	-	-	2.47	0.10	-	-
12	2.46	89.8	296.0	-	-	-	-	2.34	0.12	-	-
13	2.80	160.0	324.8	-	-	-	-	0.70	0.03	1.98	0.09
Totals	37.34			2.29	2.65	0.55	2.74	25.75	1.29	1.98	0.09
% Esters				6.13	7.10	1.47	7.34	68.97	3.45	5.30	0.24
% Acids				6.07	7.07	1.45	7.31	69.07	3.45	5.33	0.25
% Fraction (18.7)				1.14	1.32	0.27	1.36	12.91	0.65	1.00	0.05

Isomerisation Data

Fraction	I.V. (acids)	$E^{1\%}_{1cm}$ at 234mμ	$E^{1\%}_{1cm}$ at 268mμ
B9	96.2	42.7	7.7*

Isomerisation was carried out at 180°C for 60 minutes with standard procedure. U.V. absorption of unisomerised fraction was negligible. * In general, if this value is greater than 10 under these conditions, then the isomerisation is repeated at 170°C for 15 minutes.

Table 21 - Distillation of C esters from C. niloticus fat

No.	weight gm.	I.V.	S.E.	C_{14}^{0}	C_{16}^{0}	C_{14}^{1}	C_{16}^{1}
1	3.02	31.8	243.6	2.10	-	0.71	0.20
2	2.98	75.2	260.6	0.70	0.06	0.11	2.02
3	2.82	95.7	265.1	0.09	0.07	0.21	2.34
4	2.93	98.8	266.4	-	0.08	-	2.73
5	2.87	98.1	267.5	-	0.07	-	2.67
6	2.79	96.9	267.5	-	0.07	-	2.60
7	2.84	95.4	268.0	-	0.07	-	2.65
8	3.51	94.4	274.7	-	0.07	-	2.44
9	3.25	107.3	288.0	-	0.02	-	0.78
10	3.46	130.0	291.2	-	-	-	-
11	3.38	130.9	293.2	-	-	-	-
12	3.54	130.5	293.3	-	-	-	-
13	2.84	130.2	292.5	-	-	-	-
14	3.25	130.5	292.5	-	-	-	-
15	3.50	129.3	293.7	-	-	-	-
16	3.01	128.0	293.9	-	-	-	-
17	3.72	128.6	294.1	-	-	-	-
18	3.77	137.0	297.0	-	-	-	-
19	3.63	212.6	313.5	-	-	-	-
20	3.12	225.3	322.4	-	-	-	-
21	2.32	297.1	335.5	-	-	-	-
22	6.50	278.0	372.0	-	-	-	-
Totals 73.05				2.89	0.51	1.03	18.43
	% Esters			3.96	0.70	1.41	25.23
	% Acids			3.92	0.69	1.40	25.11
	% Fraction (50.5)			1.98	0.35	0.71	12.68

Isomerisation Data ($E^{1\%}_{1cm}$ values)

Fraction	I.V. (acids)	234mμ	268mμ	300mμ	315mμ	346mμ
C5	102.3	35.0	11.4*	-	-	-
C14	135.0	266.5	70.9*	-	-	-
C20	234.9	386.5	207.4	82.0	73.0	14.5
C21	307.2	409.1	231.1	123.8	116.3	44.9

* These values were measured after 170°/15 min. - the remainder after 180°/60 min. The U.V. absorptions of fractions C5 and C14 unisomerised were negligible.

C_{16}^{11}	C_{16}^{111}	C_{18}^{1}	C_{18}^{11}	C_{18}^{111}	'C_{20}'	'C_{22}'	N.S.
0.01	-	-	-	-	-	-	-
0.05	0.04	-	-	-	-	-	-
0.06	0.05	-	-	-	-	-	-
0.07	0.06	-	-	-	-	-	-
0.07	0.06	-	-	-	-	-	-
0.06	0.06	-	-	-	-	-	-
0.07	0.05	-	-	-	-	-	-
0.06	0.05	0.57	0.21	0.11	-	-	-
0.02	0.02	1.53	0.57	0.31	-	-	-
-	-	2.20	0.82	0.44	-	-	-
-	-	2.15	0.80	0.43	-	-	-
-	-	2.25	0.84	0.45	-	-	-
-	-	1.80	0.68	0.36	-	-	-
-	-	2.06	0.77	0.42	-	-	-
-	-	2.22	0.83	0.45	-	-	-
-	-	1.90	0.72	0.39	-	-	-
-	-	2.35	0.89	0.48	-	-	-
-	-	2.24	0.84	0.45	0.24	-	-
-	-	0.61	0.23	0.12	2.67	-	-
-	-	-	-	-	3.12	-	Tr
-	-	-	-	-	2.21	-	0.11
-	-	-	-	-	-	6.05	0.45
0.47	0.38	21.88	8.20	4.41	8.24	6.05	0.56
0.64	0.52	29.94	11.23	6.04	11.28	8.28	0.77
0.64	0.51	29.97	11.23	6.04	11.33	8.35	0.81
0.32	0.26	15.13	5.67	3.05	5.72	4.22	0.41

Table 22 - Component acids of C. niloticus fat

Acid	A	B	C	Total % wt.	Excluding Unsaponifiable	
					% wt.	% mol.
Saturated						
Myristic	0.79	1.14	1.98	3.91	3.93	4.69
Palmitic	22.25	1.32	0.35	23.92	24.03	25.55
Stearic	3.40	-	-	3.40	3.42	3.28
Arachidic	1.34	-	-	1.34	1.35	1.18
Unsaturated						
Tetradecenoic	-	0.27	0.71	0.98	0.98	1.18
Hexadecenoic	0.29	1.36	12.68	14.33	14.40	15.43
Hexadecadienoic	-	-	0.32	0.32	0.32	0.34
Hexadecatrienoic	-	-	0.26	0.26	0.26	0.28
Octadecenoic	2.60	12.91	15.13	30.64	30.79	29.70
Octadecadienoic	0.12	0.65	5.67	6.44	6.47	6.29
Octadecatrienoic	-	-	3.05	3.05	3.06	3.00
As 'eicosenoic' +	-	1.00	5.72	6.72	6.75	5.74
As 'docosenoic' ++	-	-	4.22	4.22	4.24	3.34
Unsaponifiable	0.01	0.05	0.41	0.47	-	-

+ Average unsaturation - 5.8H. ++ Average unsaturation - 7.9H.

In addition to the unsaturated acids discussed on page 24, the following acids were isolated from appropriate fractions of C. niloticus fat:- Myristic acid m.pt. 50-52°C.; palmitic acid m.pt. 63-64°C.; stearic acid m.pt. 68-69°C. Mixed m.pts. with authentic samples gave no depressions. Attempts to separate arachidic acid in a sufficiently pure state were unsuccessful. See also Part II (page 130).

B Analyses of Ostrich and Flamingo Fats (see page 33)

Table 23 - Characteristics of Ostrich and Flamingo Fats

	FAT		F.F.A. as oleic	I.V. of Mixed Acids
	I.V.	S.E.		
Ostrich	80.4	282.8	2.1	83.4
Flamingo	65.8	283.0	4.2	67.9

Table 24 - Fractional Crystallisation of Ostrich Fat
(corrected wts.)

Mixed Acids 197.6gm. I.V. 83.4

10 x MeOH — 40°C

Insoluble 137.5gm. I.V. 52.9

Soluble 60.1gm. I.V. 154.9 (Fraction C)

10 x MeOH — -20°C

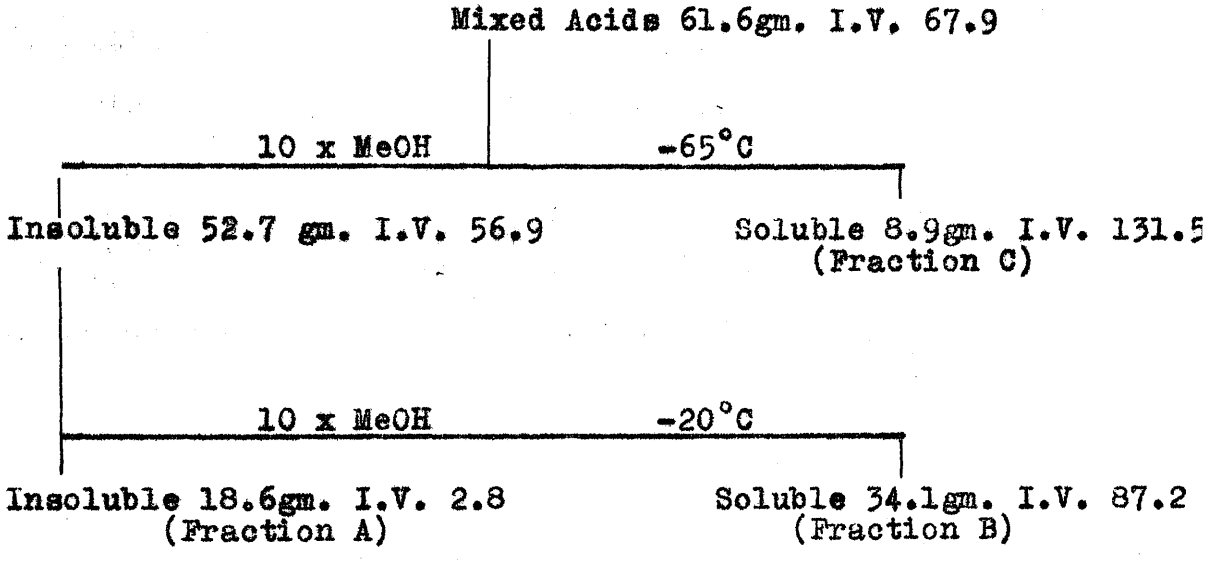
Insoluble 77.5gm. I.V. 21.1 (Fraction A)

Soluble 60.0gm. I.V. 94.4 (Fraction B)

i.e.

Fraction	A	B	C
Weight (gm.)	77.5 39.2	60.0 30.4	60.1 30.4
% wt. of total I.V.	21.1	94.4	154.9

Table 25 - Fractional Crystallisation of Flamingo Fat
(corrected wts.)



i.e.

Fraction	A	B	C
weight (gm).	18.6	34.1	8.9
% wt. of total	30.2	55.4	14.4
I.V.	2.8	87.2	131.5

Table 26 - Distillation of A esters from ostrich fat

No.	wt. gm.	I.V.	S.E.	C_{14}°	C_{16}°	C_{18}°	C_{20}°	C_{16}^{1}	C_{18}^{1}	C_{18}^{11}	N.S.
1	2.74	2.1	268.3	0.18	2.50	-	-	0.06	-	-	-
2	2.92	1.6	271.2	-	2.87	-	-	0.05	-	-	-
3	3.08	1.6	271.9	-	3.03	-	-	0.05	-	-	-
4	3.40	1.5	270.9	-	3.35	-	-	0.05	-	-	-
5	3.35	1.4	271.8	-	3.30	-	-	0.05	-	-	-
6	2.83	1.8	271.9	-	2.78	-	-	0.05	-	-	-
7	3.13	1.8	271.0	-	3.07	-	-	0.06	-	-	-
8	3.69	2.8	271.9	-	3.58	-	-	0.11	-	-	-
9	2.99	2.1	270.0	-	2.92	-	-	0.07	-	-	-
10	3.60	27.0	279.3	-	2.31	0.20	-	-	1.05	0.04	-
11	3.41	59.8	294.6	-	0.28	0.84	-	-	2.20	0.09	-
12	3.52	56.3	297.3	-	-	1.35	-	-	2.09	0.08	-
13	2.99	52.5	297.7	-	-	1.23	-	-	1.69	0.07	-
14	3.32	46.6	297.9	-	-	1.58	-	-	1.67	0.07	-
15	2.67	37.3	298.4	-	-	1.55	-	-	1.08	0.04	-
16	1.74	25.7	313.7	-	-	0.70	0.56	-	0.42	0.02	0.04
Totals	49.38			0.18	29.99	7.45	0.56	0.55	10.20	0.41	0.04
% Esters				0.36	60.74	15.09	1.13	1.11	20.66	0.83	0.08
% Acids				0.36	60.62	15.14	1.14	1.11	20.72	0.83	0.08
% Fraction (39.2)				0.14	23.76	5.93	0.45	0.44	8.12	0.33	0.03

Fraction A12 (I.V. of acids 57.5) had $E_{1cm}^{1\%}$ of 21.8 at 234mμ after isomerisation at 180°C/60 min., U.V. of unisomerised fraction being negligible.

Table 27 - Distillation of B esters from ostrich fat

No.	wt. gm.	I.V.	S.E.	C_{14}°	C_{16}°	C_{14}^{1}	C_{16}^{1}	C_{18}^{1}	C_{18}^{11}	'C$_{20}$'	N.S.
1	2.81	48.0	266.0	0.09	0.67	0.22	1.83	-	-	-	-
2	2.64	62.6	278.3	-	0.46	-	1.25	0.83	0.10	-	-
3	2.97	92.2	294.5	-	0.05	-	0.13	2.48	0.31	-	-
4	3.48	95.3	295.4	-	-	-	-	3.09	0.39	-	-
5	3.66	94.9	296.6	-	-	-	-	3.27	0.39	-	-
6	3.54	94.9	296.5	-	-	-	-	3.16	0.38	-	-
7	3.44	94.8	295.4	-	-	-	-	9.01	1.06	-	-
8	3.12	94.8	295.6	-	-	-	-			-	-
9	3.51	94.8	295.4	-	-	-	-			-	-
10	2.69	94.2	295.2	-	-	-	-	2.43	0.26	-	-
11	3.52	93.8	296.0	-	-	-	-			-	-
12	3.32	93.8	296.6	-	-	-	-	12.35	1.27	-	-
13	3.40	93.8	296.6	-	-	-	-			-	-
14	3.38	93.8	296.1	-	-	-	-			-	-
15	2.27	92.2	296.1	-	-	-	-	2.10	0.17	-	-
16	2.62	91.4	295.7	-	-	-	-	2.45	0.17	-	-
17	1.71	90.9	316.0	-	-	-	-	1.05	0.07	0.53	0.06
Totals	52.08			0.09	1.18	0.22	3.21	42.22	4.57	0.53	0.06
% Esters				0.17	2.27	0.42	6.16	81.07	8.77	1.02	0.12
% Acids				0.17	2.26	0.42	6.13	81.09	8.77	1.03	0.13
% Fraction (30.4)				0.05	0.69	0.13	1.86	24.65	2.67	0.31	0.04

Isomerisation Data ($E_{1cm}^{1\%}$ values)

Fraction	I.V. of acids	234mμ	268mμ
B2	66.0	41.3	1.0
B11	98.8	77.4	6.6

All measurements were made after isomerisation at 180°C/60 min.

Table 28 - Distillation of C esters from ostrich fat

No.	wt. gm.	I.V.	S.E.	C_{14}°	C_{16}°	C_{14}^{1}
1	2.46	65.7	244.7	0.99	0.01	1.09
2	3.06	96.1	264.5	0.18	0.11	0.19
3	2.77	116.5	272.3	-	0.09	-
4	2.92	142.6	283.4	-	0.15	-
5	3.57	153.6	287.8	-	0.08	-
6	3.03	157.0	291.1	-	-	-
7	3.49	159.1	289.4	-	-	-
8	3.48	158.9	290.0	-	-	-
9	3.11	159.3	290.8	-	-	-
10	2.93	160.1	291.9	-	-	-
11	3.21	160.3	290.7	-	-	-
12	2.80	160.0	292.0	-	-	-
13	2.99	159.9	290.7	-	-	-
14	3.16	160.3	290.5	-	-	-
15	3.30	159.8	291.6	-	-	-
16	2.16	159.3	291.6	-	-	-
17	3.44	128.9	315.0	-	-	-
Totals	51.88			1.17	0.44	1.28
	% Esters			2.27	0.85	2.47
	% Acids			2.24	0.85	2.45
% Fraction	(30.4)			0.68	0.26	0.74

Isomerisation Data ($E_{1cm}^{1\%}$ values)

Fraction	I.V. of acids	234mμ	268mμ
C1	68.5	38.2	6.8
C3	119.0	223.7	15.0
C11	165.5	592.0	84.1*

* Measured after isomerisation at 170°/15 min. - the remainder after 180°/60 min. The U.V. absorption of these fractions when unisomerised was negligible.

C_{14}^{11}	C_{16}^{1}	C_{16}^{11}	C_{18}^{1}	C_{18}^{11}	C_{18}^{111}	N.S.
0.04	0.28	0.05	-	-	-	-
0.01	2.18	0.39	-	-	-	-
-	1.87	0.34	0.13	0.27	0.07	-
-	0.86	0.04	0.52	1.06	0.29	-
-	0.46	0.02	0.84	1.70	0.47	-
-	-	-				-
-	-	-				-
-	-	-				-
-	-	-				-
-	-	-				-
-	-	-	9.36	19.08	5.22	-
-	-	-				-
-	-	-				-
-	-	-				-
-	-	-				-
-	-	-				-
-	-	-	0.88	1.81	0.49	0.26
0.05	5.65	0.84	11.73	23.92	6.54	0.26
0.10	10.89	1.62	22.60	46.10	12.61	0.50
0.09	10.85	1.61	22.63	46.14	12.62	0.53
0.03	3.30	0.49	6.88	14.03	3.83	0.16

Table 29 - Distillation of A esters from flamingo fat

No.	wt. gm.	I.V.	S.E.	C_{16}°	C_{18}°	C_{18}^{1}	N.S.
1	2.25	0	269.2	2.25	-	-	-
2	2.66	0	268.4	2.66	-	-	-
3	2.53	0	268.3	2.53	-	-	-
4	2.57	0	269.0	2.57	-	-	-
5	2.04	3.3	277.1	1.51	0.45	0.08	-
6	2.42	5.8	281.9	1.37	0.89	0.16	-
7	2.25	4.5	297.5	-	2.13	0.12	-
8	1.20	5.3	317.5	-	1.06	0.06	0.08
Totals	17.92			12.89	4.53	0.42	0.08
% Esters				71.93	25.28	2.34	0.45
% Acids				71.81	25.37	2.35	0.47
% Fraction (30.2)				21.69	7.66	0.71	0.14

Table 30 - Distillation of B esters from flamingo fat

No.	wt. gm.	I.V.	S.E.	C_{16}°	C_{16}^{1}	C_{18}^{1}	C_{18}^{11}	'C_{20}'	N.S.
1	1.63	51.0	272.0	0.74	0.72	0.17	-	-	-
2	2.38	68.5	284.0	0.53	0.51	1.34	-	-	-
3	2.26	81.3	291.1	0.14	0.27	1.85	-	-	-
4	2.70	85.8	243.8	-	-	2.70	-	-	-
5	2.46	87.3	293.7	-	-	2.41	0.05	-	-
6	2.25	87.6	294.5	-	-	2.20	0.05	-	-
7	2.25	87.5	293.9	-	-	2.20	0.05	-	-
8	2.34	88.0	294.3	-	-	2.28	0.06	-	-
9	3.34	88.3	293.8	-	-	3.24	0.10	-	-
10	2.57	87.9	293.6	-	-	2.50	0.07	-	-
11	2.47	87.3	293.4	-	-	2.42	0.05	-	-
12	2.22	87.2	293.5	-	-	2.18	0.04	-	-
13	2.00	86.6	293.7	-	-	1.98	0.02	-	-
14	1.45	80.4	332.3	-	-	0.81	0.01	0.51	0.12
Totals	32.32			1.41	1.50	28.28	0.50	0.51	0.12
% Esters				4.36	4.64	87.50	1.55	1.58	0.37
% Acids				4.34	4.62	87.51	1.55	1.59	0.39
% Fraction (55.4)				2.40	2.56	48.48	0.86	0.88	0.22

Isomerisation Data

Fraction	I.V. (acids)	$E^{1\%}_{1cm}$ at 234mμ after isomerisn. at 180°C/60 min.
B9	92.0	32.3

U.V. absorption of unisomerised fraction was negligible.

Table 31 - Distillation of C esters from flamingo fat

No.	wt. gm.	I.V.	S.E.	C_{16}°	C_{16}^{1}	C_{16}^{11}	C_{18}^{1}	C_{18}^{11}	C_{18}^{111}	'C$_{20}$'	NS.
1	0.87	93.1	269.7	0.16	0.56	0.15	-	-	-	-	-
2	1.22	117.2	277.9	0.13	0.43	0.12	0.21	0.32	0.01	-	-
3	1.20	134.8	289.7	0.03	0.12	0.03	0.39	0.61	0.02	-	-
4	1.21	138.9	294.0	-	-	-				-	-
5	0.93	138.9	292.8	-	-	-	1.38	2.14	0.08	-	-
6	1.46	136.7	294.5	-	-	-				-	-
7	1.76	122.9	325	-	-	-	0.41	0.64	0.02	0.58	0.11
Totals				0.32	1.11	0.30	2.39	3.71	0.13	0.58	0.11
% Esters				3.70	12.83	3.47	27.63	42.89	1.50	6.71	1.27
% Acids				3.69	12.77	3.45	27.64	42.88	1.50	6.74	1.33
% Fraction				0.53	1.84	0.50	3.98	6.17	0.22	0.97	0.19

Isomerisation Data ($E_{1cm}^{1\%}$ values)

Fraction	I.V. (acids)	234mμ	268mμ
C1	97.9	172.8	-
C4	145.0	540.6	12.6*

* Measured after isomerisation at 170°/15 min. - remainder at 180°C/60 min.

U.V. absorption of unisomerised fractions was negligible.

Table 32 - Component acids of ostrich fat

Acid	A	B	C	Total % wt.	Excluding Unsaponifiable % wt.	Excluding Unsaponifiable % mol.
Saturated						
Myristic	0.14	0.05	0.68	0.87	0.87	1.04
Palmitic	23.76	0.69	0.26	24.71	24.77	26.32
Stearic	5.93	-	-	5.93	5.94	5.69
Arachidic	0.45	-	-	0.45	0.45	0.39
Unsaturated						
Tetradecenoic	-	0.13	0.74	0.87	0.87	1.05
+ Tetradecadienoic	-	-	0.03	0.03	0.03	0.04
Hexadecenoic	0.44	1.86	3.30	5.60	5.61	6.01
Hexadecadienoic	-	-	0.49	0.49	0.49	0.53
Octadecenoic	8.12	24.65	6.88	39.65	39.75	38.32
Octadecadienoic	0.33	2.67	14.03	17.03	17.07	16.58
Octadecatrienoic	-	-	3.83	3.83	3.84	3.76
‡ as 'eicosenoic'	-	0.31	-	0.31	0.31	0.27
Unsaponifiable	0.03	0.04	0.16	0.23	-	-

+ This small quantity of acid seems hardly significant.

‡ Average unsaturation - 2.1H.

In addition to the acids mentioned in pages 34-37 the following acids were identified in appropriate fractions of ostrich fat:- Palmitic acid m.pt. 62°C.; Stearic acid m.pt. 68°C.; hexadecenoic acid as dihydroxy palmitic acid m.pt. 126-127°C.; oleic acid as dihydroxystearic acid m.pt. 130-131°C.; linoleic acid as tetrabromostearic acid m.pt, 111-113°C.; linolenic acid as hexabromostearic acid m.pt. 181-182°C. Mixed m.pts. with authentic samples of acids showed no depressions.

Table 33 - Component acids of flamingo fat

Acid	A	B	C	Total % wt.	Excluding Unsaponifiable	
					% wt.	% mol.
Saturated						
Palmitic	21.69	2.40	0.53	24.62	24.76	26.51
Stearic	7.66	-	-	7.66	7.70	7.43
Unsaturated						
Hexadecenoic	-	2.56	1.84	4.40	4.42	4.77
Hexadecadienoic	-	-	0.50	0.50	0.50	0.54
Octadecenoic	0.71	48.48	3.98	53.17	53.47	51.97
Octadecadienoic	-	0.86	6.17	7.03	7.07	6.92
Octadecatrienoic	-	-	0.22	0.22	0.22	0.22
+ as 'eicosenoic'	-	0.88	0.97	1.85	1.86	1.04
Unsaponifiable	0.14	0.22	0.19	0.55	-	-

+ Average unsaturation - 2.7H.

In addition to the acids discussed on pages 34-37 the following acids were identified in appropriate fractions of flamingo fat.

Palmitic acid m.pt. 62-63°C.; stearic acid m.pt. 69-70°C.; hexadecenoic acid as dihydroxypalmitic acid m.pt. 124.5-125.5°C.; oleic acid as dihydroxystearic acid m.pt. 129-130°C.; linoleic acid as tetrabromostearic acid m.pt. 112-113°C. Mixed m.pts. with authentic samples showed no depressions.

C. Analyses of Rabbit, Mouse and Porcupine Fats (see page 42)

Table 34 - Characteristics of rabbit, mouse and porcupine fats.

	FAT			I.V. of Mixed Acids
	I.V.	S.E.	F.F.A.	
Rabbit	76.9	267.9	8.4	81.1
Mouse	86.8	286.8	3.8	91.6
Porcupine	50.3	271.0	6.0	52.3

Table 35 - Fractional Crystallisation of Rabbit Fat
(corrected wts.)

Mixed Acids 111.2gm. I.V. 81.1

10 x MeOH | -55°C

Insoluble 65.6gm. I.V. 43.8

Soluble 45.6gm. I.V. 135.1 (Fraction C)

10 x MeOH | - 20°C.

Insoluble 32.3gm. I.V. 1.2 (Fraction A)

Soluble 33.3gm. I.V. 85.5 (Fraction B)

i.e.

Fraction	A	B	C
weight (gm.)	32.3	33.3	45.6
% wt. of total	29.1	29.9	41.0
I.V.	1.2	85.5	135.1

Table 36 - Fractional Crystallisation of Mouse fat

(corrected wts.)

Mixed Acids 55.0gm. I.V. 91.6

10 x MeOH -60°C

Insoluble 36.8gm. I.V. 58.5

Soluble 18.2gm. I.V. 159.6 (Fraction C)

10 x MeOH - 20°C

Insoluble 15.1gm. I.V. 1.5 (Fraction A)

Soluble 21.7gm. I.V. 97.5 (Fraction B)

i.e.

Fraction	A	B	C
weight (gm.)	15.1	21.7	18.2
% wt. of total	27.3	39.6	33.1
I.V.	1.5	97.5	159.6

Table 37 - Fractional Crystallisation of Porcupine fat

(corrected wts.)

Mixed Acids 93.8gm. I.V. 52.3

10 x MeOH -55°C

Insoluble 66.5gm. I.V. 28.0

Soluble 27.3gm. I.V. 111.2 (Fraction C)

10 x MeOH -20°C

Insoluble 43.6gm. I.V. 0.8 (Fraction A)

Soluble 22.9gm. I.V. 79.6 (Fraction B)

i.e.

Fraction	A	B	C
weight (gm.)	43.6	22.9	27.3
% wt. of total	46.5	24.4	29.1
I.V.	0.8	79.6	111.2

Table 38 - Distillation of A esters from rabbit fat

No.	wt. gm.	I.V.	S.E.	C_{14}°	C_{16}°	C_{18}°	C_{20}°	C_{18}^{1}	N.S.
1	2.31	0	268.5	0.14	2.17	-	-	-	-
2	3.43	0	270.5	-	3.43	-	-	-	-
3	3.24	0	270.7	-	3.24	-	-	-	-
4	2.73	0	269.9	-	2.73	-	-	-	-
5	3.12	0	270.5	-	3.12	-	-	-	-
6	3.38	0	270.8	-	3.38	-	-	-	-
7	3.38	0	271.4	-	3.25	0.13	-	-	-
8	2.48	0.8	273.7	-	2.17	0.29	-	0.02	-
9	3.45	2.4	290.1	-	0.96	2.39	-	0.10	-
10	2.18	3.8	296.4	-	0.14	1.94	-	0.10	-
11	1.76	4.2	313.1	-	-	1.24	0.38	0.09	0.05
Totals	31.46			0.14	24.59	5.99	0.38	0.31	0.05
	% Esters			0.45	78.15	19.04	1.21	0.99	0.16
	% Acids			0.44	78.06	19.12	1.22	0.99	0.17
	% Fraction (29.1)			0.13	22.71	5.56	0.36	0.29	0.05

Table 39 - Distillation of B esters from rabbit fat

No.	wt. gm.	I.V.	S.E.	C_{14}°	C_{16}°	C_{16}^{1}	C_{18}^{1}	C_{18}^{11}	N.S.
1	1.35	4.1	244.5	1.23	0.06	0.06	-	-	-
2	2.48	35.3	267.6	0.17	1.38	0.93	-	-	-
3	2.68	68.1	285.1	-	0.64	0.43	1.56	0.05	-
4	3.21	88.6	291.2	-	0.35	0.23	2.55	0.08	-
5	1.68	88.2	296.6	-	-	-	1.63	0.05	-
6	1.79	87.5	296.4	-	-	-	1.75	0.04	-
7	3.47	91.9	296.4	-	-	-	3.22	0.25	-
8	2.75	93.4	295.6	-	-	-	2.51	0.24	-
9	3.42	93.6	296.9	-	-	-	3.11	0.31	-
10	3.08	94.1	296.3	-	-	-	2.78	0.30	-
11	3.51	93.1	296.1	-	-	-	3.21	0.30	-
12	1.03	92.2	305.8	-	-	-	0.91	0.09	0.03
13	1.64	81.6	298.6	-	-	-	1.44	0.14	0.06
Totals	32.09			1.40	2.43	1.65	24.67	1.85	0.09
	% Esters			4.36	7.57	5.14	76.88	5.77	0.28
	% Acids			4.32	7.54	5.12	76.96	5.77	0.29
	% Fraction (29.9)			1.29	2.25	1.53	23.01	1.73	0.09

Isomerisation Data

Fraction	I.V. of acids	$E^{1\%}_{1cm}$ at 234mμ after isom$^{n.}$ at 180°C/ 60 min.
B9	97.3	84.2

U.V. absorption of unisomerised fraction was negligible.

Table 40 - Distillation of C esters from rabbit fat

No.	wt. gm.	I.V.	S.E.	C_{14}°	C_{14}^{1}	C_{16}^{1}	'C_{18}'+	N.S.
1	2.68	55.5	199.4	0.94	1.72	-	-	0.02
2	2.87	85.3	258.0	0.36	0.66	1.85	-	-
3	2.64	113.9	273.4	-	-	2.09	0.55	-
4	2.64	145.8	282.9	-	-	0.64	2.00	-
5	3.55	159.3	289.2	-	-	0.15	3.40	-
6	3.06	162.6	290.3	-	-	-	3.06	-
7	2.97	162.8	291.2	-	-	-	2.97	-
8	3.10	162.5	291.5	-	-	-	3.10	-
9	3.42	161.4	291.2	-	-	-	3.42	-
10	3.45	162.5	291.2	-	-	-	3.45	-
11	3.44	161.6	292.8	-	-	-	3.44	-
12	3.22	145.8	292.9	-	-	-	3.22	-
13	2.00	132.2	280.6	-	-	-	2.00	-
14	4.63	83.5	320	-	-	-	4.25	0.38
Totals	43.67			1.30	2.38	4.73	34.86	0.40
% Esters				2.98	5.45	10.83	79.82	0.92
% Acids				2.95	5.39	10.79	79.90	0.97
% Fraction (41.0)				1.21	2.21	4.42	32.76	0.40

Isomerisation Data ($E_{1cm}^{1\%}$ values)

Fraction	I.V. of Acids	234mμ	268mμ
C3	121.3	278.3	28.0*
C8	170.7	693.6	85.5*

* These values were measured after isomerisation at 170°C/15 min. remainder after 180°C/60 min.
U.V. absorption of unisomerised fractions was negligible.

\+ Using spectral and iodine values the percentages of C_{18} acids do not sum to approximately 100% (see pages 15,44), probably because of deterioration. Using these values however the 'C_{18}' fraction has the following composition:-

C_{18}^{1} 9.6%: C_{18}^{11} 73.5%: C_{18}^{111} 16.9%. (by interpolation to 100%)

Table 41 - Distillation of A esters from mouse fat

No.	wt. gm.	I.V.	S.E.	C_{16}°	C_{18}°	C_{18}^{1}	N.S.
1	2.37	0	269.5	2.37	-	-	-
2	1.95	0	270.0	1.95	-	-	-
3	2.27	0	269.5	2.27	-	-	-
4	1.68	0	269.8	1.68	-	-	-
5	1.53	0	269.4	1.53	-	-	-
6	1.65	0	269.5	1.65	-	-	-
7	1.58	6.8	281.4	0.92	0.53	0.13	-
8	1.15	11.7	310.5	0.13	0.78	0.16	0.08
Totals	14.20			12.50	1.31	0.29	0.08
	% Esters			88.15	9.24	2.05	0.56
	% Acids			88.08	9.28	2.05	0.59
	% Fraction (27.3)			24.14	2.54	0.56	0.16

Table 42 - Distillation of B esters from mouse fat

No.	wt. gm.	I.V.	S.E.	C_{14}°	C_{16}°	C_{16}^{1}	C_{18}^{1}	C_{18}^{11}	'C_{20}'	N.S.
1	1.87	50.8	267.6	0.10	0.77	1.00	-	-	-	-
2	1.88	82.6	288.9	-	0.23	0.29	1.23	0.13	-	-
3	1.83	93.7	296.3	-	-	-	1.66	0.17	-	-
4	2.07	96.4	295.2	-	-	-	1.81	0.26	-	-
5	2.24	98.7	294.4	-	-	-	1.91	0.33	-	-
6	2.23	99.8	295.0	-	-	-	1.87	0.36	-	-
7	2.42	99.1	295.3	-	-	-	2.05	0.37	-	-
8	1.81	98.8	296.7	-	-	-	1.54	0.27	-	-
9	2.22	99.6	297.4	-	-	-	1.80	0.32	0.10	-
10	2.33	90.5	320.1	-	-	-	1.52	0.27	0.40	0.14
Totals	20.90			0.10	1.00	1.29	15.39	2.48	0.50	0.14
	% Esters			0.48	4.78	6.17	73.64	11.87	2.39	0.67
	% Acids			0.47	4.76	6.14	73.67	11.86	2.40	0.70
	% Fraction (39.6)			0.19	1.88	2.43	29.09	4.68	0.95	0.28

Isomerisation Data

Fraction	I.V. of Acids	$E^{1\%}_{1cm}$ at 234mμ after isom^{n}. at 180°C/ 60 min.
B6	103.0	148.8

U.V. absorption of unisomerised fraction was negligible.

Table 43 - Distillation of C esters from mouse fat

No.	wt. gm.	I.V.	S.E.	C_{16}°	C_{16}^{1}	C_{18}^{1}	C_{18}^{11}	C_{18}^{111}	N.S.
1	1.88	110.5	233.3	0.17	1.03	0.14	0.50	0.04	-
2	1.98	134.0	283.1	0.09	0.57	0.27	0.97	0.08	-
3	2.34	157.0	290.8	-	-				-
4	2.57	158.1	290.8	-	-				-
5	2.71	159.9	294.1	-	-	2.56	9.24	0.81	-
6	2.32	159.0	295.3	-	-				-
7	0.95	164.3	294.8	-	-				-
8	1.91	133.1	~330	-	-				0.19
Totals	16.66			0.26	1.60	2.97	10.71	0.93	0.19
		% Esters		1.56	9.60	17.83	64.29	5.58	1.14
		% Acids		1.55	9.56	17.84	64.27	5.58	1.20
		% Fraction (33.1)		0.51	3.16	5.91	21.27	1.85	0.40

Isomerisation Data ($E_{1cm}^{1\%}$ values)

Fraction	I.V. of acids	234mμ	268mμ
C1	116.5	247.8	-
C2	139.8	520.4	24.7*
C4	164.1	683.4	34.2*
C5	164.3	684.5	37.9*

*These values were measured after isomerisation at 170°C/15 min., the remainder after 180°C/60 min.

Table 44 - Distillation of A esters from porcupine fat

No.	wt. gm.	I.V.	S.E.	C_{14}°	C_{16}°	C_{18}°	C_{18}^{1}	N.S.
1	1.91	0	263.3	0.44	1.47	-	-	-
2	2.80	0	269.0	0.12	2.68	-	-	-
3	3.39	0	270.3	-	3.39	-	-	-
4	2.98	0	270.6	-	2.98	-	-	-
5	2.77	0	270.9	-	2.77	-	-	-
6	3.00	0	271.3	-	3.00	-	-	-
7	3.30	0	271.2	-	3.30	-	-	-
8	2.38	0	270.6	-	2.38	-	-	-
9	2.60	0	270.2	-	2.60	-	-	-
10	2.77	0	270.5	-	2.77	-	-	-
11	2.83	0.7	274.9	-	2.35	0.46	0.02	-
12	2.01	1.7	284.6	-	0.94	1.03	0.04	-
13	3.00	2.2	294.1	-	0.43	2.49	0.08	-
14	3.02	1.9	294.7	-	0.38	2.57	0.07	-
15	2.51	1.5	296.5	-	0.16	2.31	0.04	-
16	2.12	3.9	314.0	-	-	1.96	0.04	0.12
Totals	43.39			0.56	31.60	10.82	0.29	0.12
	% Esters			1.29	72.82	24.94	0.67	0.28
	% Acids			1.28	72.73	25.03	0.67	0.29
	% Fraction (46.5)			0.60	33.82	11.64	0.31	0.13

Table 45 - Distillation of B esters from porcupine fat

No.	wt. gm.	I.V.	S.E.	C_{14}°	C_{16}°	C_{14}^{1}	C_{16}^{1}	C_{18}^{1}	C_{18}^{11}	N.S.
1	1.85	9.7	244.8	1.58	0.09	0.09	0.09	-	-	-
2	2.47	45.7	270.2	-	1.28	-	1.19	-	-	-
3	2.26	78.5	287.2	-	0.24	-	0.23	1.75	0.04	-
4	2.54	87.2	293.7	-	-	-	-	2.49	0.05	-
5	2.82	89.8	295.1	-	-	-	-	2.68	0.14	-
6	2.88	90.8	294.2	-	-	-	-	2.71	0.17	-
7	2.56	89.7	294.7	-	-	-	-	2.44	0.12	-
8	3.08	85.9	293.5	-	-	-	-	3.07	0.01	-
9	1.47	75.2	308.5	-	-	-	-	1.37	-	0.10
Totals	21.93			1.58	1.61	0.09	1.51	16.51	0.53	0.10
	% Esters			7.20	7.34	0.41	6.89	75.28	2.42	0.46
	% Acids			7.13	7.31	0.41	6.86	75.39	2.42	0.48
	% Fraction (24.4)			1.75	1.79	0.10	1.68	18.47	0.59	0.12

A sample of B6 esters when isomerised at 180°C/60 min. had an $E_{1cm}^{1\%}$ of 38.8, the U.V. absorption of the unisomerised esters being negligible (at 234 mμ).

Table 46 - Distillation of C esters from porcupine fat

No.	wt. gm.	I.V.	S.E.	C_{14}°	C_{16}°	C_{14}^{1}	C_{16}^{1}	C_{16}^{11}	C_{18}^{1}	C_{18}^{11}	C_{18}^{111}	N.S
1	1.88	17.1	208.7	1.58	-	0.30	-	-	-	-	-	-
2	1.84	60.1	243.4	0.78	0.03	0.90	0.10	0.03	-	-	-	-
3	2.35	103.4	272.1	-	0.30	-	1.31	0.34	0.15	0.24	0.01	-
4	2.22	131.3	290.1	-	0.05	-	0.19	0.05	0.73	1.16	0.04	-
5	2.13	136.6	291.9	-	-	-	-	-				-
6	2.51	137.1	293.2	-	-	-	-	-				-
7	2.68	135.6	292.7	-	-	-	-	-				-
8	2.27	135.1	291.9	-	-	-	-	-	4.94	7.85	0.29	-
9	2.08	128.1	291.8	-	-	-	-	-				-
10	1.41	108.8	295.2	-	-	-	-	-				-
11	3.03	77.4	300	-	-	-	-	-	1.01	1.61	0.06	0.35
Totals	24.40			2.36	0.38	1.20	1.60	0.42	6.83	10.86	0.40	0.35
% Esters				9.67	1.56	4.92	6.56	1.72	27.99	44.51	1.64	1.43
% Acids				9.58	1.56	4.87	6.54	1.71	28.04	44.56	1.64	1.50
% Fraction (29.1)				2.78	0.45	1.41	1.90	0.50	8.13	12.91	0.48	0.44

Isomerisation Data ($E_{1cm}^{1\%}$ values)

Fraction	I.V. of acids	234mμ	268mμ
C3	108.1	235.9	-
C6	143.0	535.5	11.5*

* Value after isomerisation for 170°C/15 min. - remainder after 180°C/60 min. U.V. absorption of unisomerised samples was negligible.

Table 47 - Component Acids of Rabbit Fat

Acid	A	B	C	Total % wt.	Excluding Unsaponifiable % wt.	Excluding Unsaponifiable % mol.
Saturated						
Myristic	0.13	1.29	1.21	2.63	2.64	3.12
Palmitic	22.71	2.25	-	24.96	25.10	26.46
Stearic	5.56	-	-	5.56	5.59	5.31
Arachidic	0.36	-	-	0.36	0.36	0.31
Unsaturated						
Tetradecenoic	-	-	2.21	2.21	2.22	2.65
Hexadecenoic	-	1.53	4.42	5.95	5.98	6.35
Octadecenoic	0.29	23.01	-	23.30	23.43	22.41
Octadecadienoic	-	1.73	-	1.73	1.74	1.68
'C_{18}' acids +	-	-	32.76	32.76	32.94	31.71
Unsaponifiable	0.05	0.09	0.40	0.54	-	-

+ Average unsaturation -3.7H. Assuming that these acids have a C_{18} content as shown in Table 40 (footnote) then the C_{18} acids of rabbit fat are given by C_{18}^{1} 26.59%; C_{18}^{11} 25.95%; C_{18}^{111} 5.57% % wt. (excluding unsaponifiable). Otherwise, the C_{18} acids (in general) have an average unsaturation of -3.0H.

The following acids were identified in appropriate fractions of rabbit fat:- Palmitic acid m.pt. 62-62.5°C.; stearic acid m.pt. 68-69°C.; hexadecenoic acid as dihydroxypalmitic acid m.pt. 123-124°C.; oleic acid as dihydroxystearic acid m.pt. m.pt. 128.5-129°C.; linoleic acid as tetrabromostearic acid m.pt. 112-113°C.; linolenic acid as hexabromostearic acid m.pt. 183-184°C.

On carrying out a quantitative bromination on a fraction rich in C_{18} polyethenoid acids, the weights of polybromides obtained indicated 23.0% linolenic and 68.0% linoleic acids. This compares with the quantities obtained by the isomerisation procedure viz. 15.4% for octadecatrienoic and 66.8% for octadecadienoic acids. These figures, bearing in mind that this fraction (C) showed some deterioriation, probably imply that these acids exist mainly as linolenic and linoleic acids.

Table 48 - Component acids of mouse fat

Acid	A	B	C	Total % wt.	Excluding Unsaponifiable % wt.	% mol.
Saturated						
Myristic	-	0.19	-	0.19	0.19	0.23
Palmitic	24.14	1.88	0.51	26.53	26.75	28.48
Stearic	2.54	-	-	2.54	2.56	2.46
Unsaturated						
Hexadecenoic	-	2.43	3.16	5.59	5.64	6.05
Octadecenoic	0.56	29.09	5.91	35.56	35.86	34.66
Octadecadienoic	-	4.68	21.27	25.95	26.17	25.48
Octadecatrienoic	-	-	1.85	1.85	1.87	1.83
as 'eicosenoic'+	-	0.95	-	0.95	0.96	0.81
Unsaponifiable	0.16	0.28	0.40	0.84	-	-

+ average unsaturation -2.2H

The following acids were identified in appropriate fractions of mouse fat:- Palmitic acid m.pt. 62-63°C.; stearic acid m.pt. 68-69°C.; hexadecenoic acid as dihydroxypalmitic acid m.pt. 125-126°C.; oleic acid as dihydroxystearic acid m.pt. 130-131°C. linoleic acid as tetrabromostearic acid m.pt. 112.5-113.5°C.; attempts to prepare a pure sample of hexabromostearic acid were unsuccessful. A quantitative bromination on a suitable fraction indicated 6.6% linolenic acid and 68% linoleic acid, compared with 6.8% and 71% as calculated from the isomerisation data - figures which indicate the possible identity of the C_{18} polyethenoid acids with linoleic and linolenic acids.

Table 49 - Component acids of porcupine fat

Acid	A	B	C	Total % wt.	Excluding Unsaponifiable	
					% wt.	% mol.
Saturated						
Myristic	0.60	1.75	2.78	5.13	5.17	6.04
Palmitic	33.82	1.79	0.45	36.06	36.32	37.84
Stearic	11.64	-	-	11.64	11.72	11.00
Unsaturated						
Tetradecenoic	-	0.10	1.41	1.51	1.52	1.79
Hexadecenoic	-	1.68	1.90	3.58	3.60	3.78
Hexadecadienoic	-	-	0.50	0.50	0.50	0.53
Octadecenoic	0.31	18.47	8.13	26.91	27.10	25.62
Octadecadienoic	-	0.59	12.91	13.50	13.59	12.94
Octadecatrienoic	-	-	0.48	0.48	0.48	0.46
Unsaponifiable	0.13	0.12	0.44	0.69	-	-

The following acids were identified in suitable fractions of porcupine fat:- Palmitic acid m.pt. 61-62°C.; stearic acid m.pt. 69.5-71°C.; hexadecenoic acid as dihydroxypalmitic acid m.pt. 124.5-125.5°C.; oleic acid as dihydroxystearic acid m.pt. 130-131°C.; linoleic acid as tetrabromostearic acid m.pt. 113-114°C.

Bromination of a fraction containing a large quantity of C_{18} polyethenoid acids was carried out in the usual way (Markley 1947). No hexabromides were deposited and the tetrabromides indicated 41.5% linoleic acid, as compared with the figure of 60.0% derived from the isomerisation procedure, suggesting that other isomeric octadecadienoic acids are present besides linoleic acid. A fraction whose $E^{1\%}_{1cm}$ value, I.V. and S.E. indicated an appreciable quantity of a hexadecadienoic acid, on bromination only deposited a tetrabromostearic acid, thus casting further doubt on the existence of a hexadecadienoic acid (cf. page 34). In all these acid identifications mixed m.pts. with authentic specimens produced no observed depressions

D - Analysis of Antelope Fat (see page 50)

Table 50 Fractional Crystallisation of antelope fat
(corrected wts.)

Mixed Acids 121.2gm. I.V.87.8

10 x MeOH -60°C

Insoluble 73.1gm. I.V. 52.7

Soluble 48.1gm. I.V. 140.4 (Fraction C)

10 x MeOH -20°C

Insoluble 30.8gm. I.V. 4.6 (Fraction A)

Soluble 42.3gm. I.V. 87.5 (Fraction B)

i.e.

Fraction	A	B	C
weight (gm.)	30.8	42.3	48.1
% wt. of total	25.4	34.9	39.7
I.V.	4.6	87.5	140.4

Table 51 - Distillation of A esters from antelope fat

No.	wt. gm.	I.V.	S.E.	C_{14}°	C_{16}°	C_{18}°	C_{20}°	C_{16}^{1}	C_{18}^{1}	N.S.
1	2.39	0.3	262.1	0.65	1.73	-	-	0.01	-	-
2	3.28	0.3	269.5	0.09	3.18	-	-	0.01	-	-
3	3.21	0.3	270.2	-	3.20	-	-	0.01	-	-
4	3.33	0.3	270.0	-	3.32	-	-	0.01	-	-
5	2.97	0.3	270.9	-	2.96	-	-	0.01	-	-
6	3.61	0.3	270.9	-	3.60	-	-	0.01	-	-
7	3.11	1.1	271.4	-	2.99	0.08	-	-	0.04	-
8	2.09	6.6	279.8	-	1.34	0.59	-	-	0.16	-
9	2.88	18.5	294.8	-	0.31	1.95	-	-	0.62	-
10	2.91	13.4	324.7	-	-	1.81	0.44	-	0.46	0.20
Totals	29.78			0.74	22.63	4.43	0.44	0.06	1.28	0.20
	% Esters			2.48	75.99	14.88	1.48	0.20	4.30	0.67
	% Acids			2.46	75.88	14.93	1.50	0.20	4.32	0.71
	% Fraction (25.4)			0.62	19.28	3.79	0.38	0.05	1.10	0.18

Table 52 - Distillation of B esters from antelope fat

No.	wt. gm.	I.V.	S.E.	C_{14}^{0}	C_{16}^{0}	C_{14}^{1}	C_{16}^{1}	C_{18}^{1}	C_{18}^{11}	N.S.
1	2.20	41.1	255.8	0.63	0.65	0.39	0.53	-	-	-
2	2.53	72.9	285.3	-	0.51	-	0.41	1.52	0.09	-
3	2.91	86.2	290.5	-	0.14	-	0.12	2.50	0.15	-
4	4.00	90.5	295.1	-	-	-	-	3.78	0.22	-
5	3.35	90.4	296.2	-	-	-	-	3.17	0.18	-
6	2.95	90.5	296.2	-	-	-	-	2.78	0.17	-
7	2.89	91.3	296.1	-	-	-	-	2.70	0.19	-
8	3.47	90.8	296.1	-	-	-	-	3.26	0.21	-
9	2.84	91.0	294.9	-	-	-	-	2.66	0.18	-
10	2.64	90.9	296.4	-	-	-	-	2.48	0.16	-
11	3.11	90.2	296.1	-	-	-	-	2.95	0.16	-
12	3.27	89.2	296.4	-	-	-	-	4.80	0.21	-
13	1.96	73.9	327.4	-	-	-	-			0.22
Totals	38.12			0.63	1.30	0.39	1.06	32.60	1.92	0.22
% Esters				1.65	3.41	1.02	2.78	85.52	5.04	0.58
% Acids				1.63	3.39	1.01	2.76	85.56	5.04	0.61
% Fraction (34.9)				0.57	1.18	0.35	0.96	29.87	1.76	0.21

A sample of B8 esters after isomerisation at 180°C/60 min. had $E_{1cm}^{1\%}$ 45.5, the U.V. absorption of the unisomerised esters being very small (at 234mμ).

Table 53 - Distillation of C esters from antelope fat

No.	wt. gm.	I.V.	S.E.	C_{14}°	C_{16}^{1}	C_{16}^{11}	C_{18}^{1}	C_{18}^{11}	C_{18}^{111}	N.S.
1	1.94	37.2	238.4	1.20	0.72	0.02	-	-	-	-
2	2.80	74.7	258.1	0.66	2.07	0.07	-	-	-	-
3	3.31	97.6	266.5	-	3.21	0.10	-	-	-	-
4	6.69	119.6	280.2	-	2.47	0.08	1.25	2.38	0.51	-
5	3.28	155.4	291.5	-	-	-				-
6	3.05	153.8	292.4	-	-	-				-
7	3.37	154.1	292.6	-	-	-				-
8	2.57	154.4	292.0	-	-	-				-
9	3.16	150.1	291.8	-	-	-				-
10	3.00	150.4	292.9	-	-	-	9.18	17.43	3.70	-
11	3.46	150.8	293.5	-	-	-				-
12	3.09	150.9	295.4	-	-	-				-
13	1.59	151.7	295.7	-	-	-				-
14	4.17	154.7	333.9	-	-	-				0.43
Totals	45.48			1.86	8.47	0.27	10.43	19.81	4.21	0.43
% Esters				4.09	18.62	0.59	22.93	43.56	9.26	0.95
% Acids				4.05	18.55	0.59	22.97	43.58	9.26	1.00
% Fraction (39.7)				1.61	7.36	0.23	9.12	17.30	3.68	0.40

Isomerisation Data ($E_{1cm}^{1\%}$ values)

Fraction	I.V. acids	234mμ	268mμ
C4	124.8	330.8	-
C8	157.3	587.9	66.5*

* Measured after isomerisation at 170°C/15min. - remainder after 180°C/60min. The U.V. absorption of the unisomerised fractions was negligible.

Table 54 - Component acids of antelope fat

Acid	A	B	C	Total % wt.	Excluding Unsaponifiable	
					% wt.	% mol.
Saturated						
Myristic	0.62	0.57	1.61	2.80	2.82	3.36
Palmitic	19.28	1.18	-	20.46	20.62	21.85
Stearic	3.79	-	-	3.79	3.82	3.65
Arachidic	0.38	-	-	0.38	0.38	0.33
Unsaturated						
Tetradecenoic	-	0.35	-	0.35	0.35	0.42
Hexadecenoic	0.05	0.96	7.36	8.37	8.44	9.02
Hexadecadienoic	-	-	0.23	0.23	0.23	0.25
Octadecenoic	1.10	29.87	9.12	40.09	40.42	38.88
Octadecadienoic	-	1.76	17.30	19.06	19.21	18.62
Octadecatrienoic	-	-	3.68	3.68	3.71	3.62
Unsaponifiable	0.18	0.21	0.40	0.79	-	-

The following acids were identified in appropriate fractions of antelope fat:- Palmitic acid m.pt. 62-63°C.; stearic acid m.pt. 68-69°C.; oleic acid as dihydroxystearic acid m.pt. 129.5-130.5°C.; hexadecenoic acid as dihydroxypalmitic acid m.pt. 126.5-127.5°C.; linoleic acid as tetrabromostearic acid 112-113°C.; linolenic acid as hexabromostearic acid m.pt. 182-183°C.. Quantitative bromination showed the presence of 3.2% linoleic and 3.0% linolenic acid in contrast to the values of 57.0% and 6.4% obtained by isomerisation procedures for octadecadi- and- trienoic acids respectively. These results suggest that the C_{18} polyethenoid acids of antelope fat are not essentially linoleic and linolenic acids.

E Analysis of Sea Lion Oil (see page 57)

Table 55 - Fractional Crystallisation of Sea Lion Oil
(corrected weights)

Mixed Acids 201.7gm. I.V. 179.3

10 x MeOH -60°C

Insoluble 122.3gm. I.V. 94.4

Soluble 79.4gm. I.V. 309.0
(Fraction C)

10 x MeOH -30°C

Insoluble 69.2 gm. I.V. 56.3
(Fraction A)

Soluble 53.1gm. I.V. 142.7
(Fraction B)

i.e.

Fraction	A	B	C
weight (gm).	69.2	53.1	79.4
% wt. of total	34.3	26.3	39.4
I.V.	56.3	142.7	309.0

Table 56 - Distillation of A esters of sea lion oil

No.	wt. gm.	I.V.	S.E.	C_{14}°	C_{16}°	C_{18}°	C_{14}^{1}	C_{16}^{1}	'C_{18}'+	'C_{20}'‡	N.S.
1	2.66	2.7	242.4	2.59	-	-	0.07	-	-	-	-
2	2.75	12.8	259.0	0.92	1.52	-	0.02	0.27	-	-	-
3	3.28	14.5	268.7	-	2.78	-	-	0.50	-	-	-
4	3.05	12.9	268.5	-	2.63	-	-	0.42	-	-	-
5	2.81	11.7	269.0	-	2.46	-	-	0.35	-	-	-
6	3.33	10.0	269.3	-	2.98	-	-	0.35	-	-	-
7	3.26	9.6	269.2	-	2.93	-	-	0.33	-	-	-
8	2.72	19.5	274.7	-	1.90	0.10	-	0.21	0.51	-	-
9	2.85	47.4	284.0	-	1.07	0.26	-	0.12	1.40	-	-
10	3.72	72.3	294.3	-	0.15	0.56	-	0.02	2.99	-	-
11	2.98	74.3	295.6	-		0.47	-	-	2.51	-	-
12	3.00	73.7	295.9	-		0.51	-	-	2.49	-	-
13	3.23	71.7	296.3	-		0.62	-	-	2.61	-	-
14	3.34	68.1	296.3	-		0.78	-	-	2.56	-	-
15	1.74	64.1	296.4	-		0.49	-	-	1.25	-	-
16	1.11	77.4	303.3	-		0.23	-	-	0.57	0.31	-
17	5.50	15L1	327.7	-		-	-	-	-	5.45	0.05
Totals	51.31			3.51	18.42	4.02	0.09	2.57	16.89	5.76	0.05
	% Esters			6.84	35.89	7.83	0.18	5.01	32.92	11.23	0.10
	% Acids			6.77	35.80	7.85	0.18	5.00	32.99	11.30	0.11
% Fraction (34.3)				2.32	12.27	2.69	0.06	1.72	11.32	3.88	0.04

+ average unsaturation - 2.1H. ‡ average unsaturation - 3.8H

Isomerisation Data

Fraction A11 (IV of acids 78.1) after isomerisation with 21% potassium hydroxide in ethylene glycol at 180°C/15min. had $E_{1cm}^{1\%}$ of 14.3 and 9.8 at 234 and 268mμ respectively, the U.V. absorption of the unisomerised fraction being negligible.

Table 57 - Distillation of B esters from sea lion oil

No.	wt. gm.	I.V.*	S.E.	C_{14}°	C_{16}°	C_{14}^{1}	'C_{16}'[1.]	'C_{18}'[2.]	'C_{20}'[3.]	'C_{22}'[4.]	N.S.
1	2.51	22.7	241.0	1.97	-	0.54	-	-	-	-	-
2	2.95	70.6	258.5	0.74	0.16	0.20	1.85	-	-	-	-
3	2.84	92.8	267.4	-	0.22	-	2.62	-	-	-	-
4	3.45	89.6	273.9	-	0.20	-	2.34	0.91	-	-	-
5	2.76	92.4	286.1	-	0.06	-	0.70	2.00	-	-	-
6	3.37	92.2	292.2	-	0.02	-	0.18	3.17	-	-	-
7	3.04	92.9	293.9	-	-	-	-	3.04	-	-	-
8	3.08	93.1	293.6	-	-	-	-	3.08	-	-	-
9	4.08	92.9	294.7	-	-	-	-	4.08	-	-	-
10	3.39	93.2	295.6	-	-	-	-	3.39	-	-	-
11	2.94	95.3	296.6	-	-	-	-	2.90	0.04	-	-
12	2.91	147.3	299.4	-	-	-	-	1.84	1.07	-	-
13	3.31	214.3	315.6	-	-	-	-	0.60	2.71	-	-
14	2.82	237.0	326.3	-	-	-	-	-	2.24	0.57	0.01
15	6.53	276.5	330.9	-	-	-	-	-	4.27	2.21	0.05
Totals	49.98			2.71	0.66	0.74	7.69	25.01	10.33	2.78	0.06
	% Esters			5.42	1.32	1.48	15.39	50.04	20.67	5.56	0.12
	% Acids			5.37	1.31	1.46	15.32	50.05	20.76	5.60	0.13
% Fraction (26.3)				1.41	0.34	0.38	4.03	13.17	5.47	1.47	0.03

* Wij's 1 hour.
1. Average unsaturation - 2.1H
2. Average unsaturation - 2.2H
3. Average unsaturation - 6.1H
4. Average unsaturation - 9.6H

Isomerisation Data ($E_{1cm}^{1\%}$ values)

Fraction	I.V. of acids	234mμ	268mμ	315mμ	346mμ
B3	94.7	27.6 (3.4)	9.1 (0.5)	7.4 (0)	-
B8	97.1	35.4 (5.0)	18.1 (0.6)	9.8 (0)	4.7 (0)
B13	216.4	239.0 (18.0)	252.3 (4.4)	151.0 (0)	65.1 (0)

All these values were measured after isomerisation with 21% KOH/glycol at 180°C/15 min. Consistent results were difficult to obtain because of turbidity (see page 69). Figures in parentheses indicate absorption when unisomerised.

Table 58 - Distillation of C esters from sea lion oil

No.	wt. gm.+	I.V.	S.E.	1. $'C_{14}'$	2. $'C_{16}'$	3. $'C_{18}'$	4. $'C_{20}'$	5. $'C_{22}'$	N.S.
1	2.56	96.7	243.9	2.23	0.33	-	-	-	-
2	2.80	125.2	263.3	0.41	2.39	-	-	-	-
3	3.55	124.0	264.4	0.39	3.16	-	-	-	-
4	3.13	125.3	266.4	0.14	2.99	-	-	-	-
5	3.23	148.4	281.5	-	1.48	1.75	-	-	-
6	3.21	161.2	289.2	-	0.56	2.65	-	-	-
7	3.38	161.9	291.1	-	0.37	3.01	-	-	-
8	3.37	262.4	302.8	-	-	2.06	1.31	-	-
9	2.73	316.7	307.2	-	-	1.15	1.58	-	-
10	3.04	351.1	312.7	-	-	0.58	2.46	-	-
11	2.98	365.1	317.2	-	-	0.03	2.95	-	-
12	3.21	373.3	322.4	-	-	-	2.56	0.65	-
13	3.52	378.3	324.0	-	-	-	2.70	0.81	0.01
14	3.42	384.6	328.0	-	-	-	2.10	1.31	0.01
15	3.27	383.3	332.2	-	-	-	1.49	1.77	0.01
16	3.45	386.6	333.4	-	-	-	1.42	2.02	0.01
17	3.04	392.9	334.3	-	-	-	1.16	1.87	0.01
18	3.22	381.1	333.4	-	-	-	1.33	1.88	0.01
19	3.06	382.0	334.8	-	-	-	1.11	1.94	0.01
20	2.78	360.2	336.7	-	-	-			
21	1.04	355.1	332.4	-	-	-	2.81	6.59	0.14
22	5.72	228.7	336.3	-	-	-			
Totals	69.71			3.17	11.28	11.23	24.98	18.84	0.21
	% Esters			4.45	16.18	16.11	35.83	27.03	0.30
	% Acids			4.50	16.06	16.07	35.89	27.17	0.31
% Fraction (39.4)				1.77*	6.33	6.33	14.15	10.70	0.12

+ Wij's 1 hour.

1. Average unsaturation -1.7H. 2. Average unsaturation -2.7H.
3. Average unsaturation -4.3H. 4. Average unsaturation -9.1H.
5. Average unsaturation -10.8H. * i.e. 0.23% C_{14}°: 1.54% C_{14}[1].

Isomerisation Data ($E^{1\%}_{1cm}$ values)

Fraction	I.V. acids	234mμ	268mμ	300mμ	315mμ	346mμ
C3	124.9	110.4(7.6)	58.9(0.8)	64.5(0)	55.5(0)	3.5(0)
C7	161.5	273.1(31.0)	136.6(3.7)	117.8(0)	106.9(0)	15.2(0)
C11	357.2	363.0(50.7)	330.1(18.3)	-	437.3(4.6)	343.2(0.5)
C17	405.5	413.6(59.7)	414.8(32.1)	320.8(6.0)	375.9(8.6)	324.1(0.6)

All these results were obtained after isomerisation with 21% KOH/glycol at 180°C for 15 min. Figures in parentheses indicate U.V. absorption values when unisomerised.

Table 59 - Component acids of sea lion oil

Acid	A	B	C	Total % wt.	Excluding Unsaponifiable % wt.	Excluding Unsaponifiable % mol.
Saturated						
Myristic	2.32	1.41	0.23	3.96	3.97	4.88
Palmitic	12.27	0.34	-	12.61	12.63	13.83
Stearic	2.69	-	-	2.69	2.70	2.66
Unsaturated						
C_{14} (-2.0H)	0.06	0.38	1.54	1.98	1.98	2.46
C_{16} (-2.4H)	1.72	4.03	6.33	12.08	12.10	13.37
C_{18} (-2.6H)	11.32	13.17	6.33	30.82	30.89	30.76
C_{20} (-7.5H)	3.88	5.47	14.15	23.50	23.54	21.67
C_{22} (-10.7H)	-	1.47	10.70	12.17	12.19	10.37
Unsaponifiable	0.04	0.03	0.12	0.19	-	-

Figures in parentheses indicate mean unsaturations.

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Part II : Separation and Identification of Unsaturated Acids.

Introduction

It has been remarked in Part I that investigations of vegetable fats have been much more extensive than those on animal fats. Thus, the structures of many acids that are characteristic of vegetable fats have been determined with a good deal of accuracy (e.g. Part III). However, in animal fats, the information on the structure of the unsaturated acids is not nearly so comprehensive and in many analyses only the average unsaturation of a group of acids is recorded. This deficiency in our knowledge of animal fats, as compared to the vegetable fats, is almost entirely due to the relative complexity of the unsaturated acids present in animal fats. The complicated mixtures of C_{20-22} unsaturated acids characteristic of marine animal oils will illustrate this point. Difficulties in the characterisation of unsaturated acids are increased both by the thermal instability of the acids and by the intricate task of separating the acids which may be of the same chain length, but differing only in the positions and configurations of the unsaturated centres, and since an unambiguous result requires the separation of the acid free of all major impurities, the difficulty of analysis will be appreciated.

Many methods of separating unsaturated acids have been tried, and chromatographic procedures seem to be yielding

the best results, although further development is required (e.g. Hammond and Lundberg 1953). [For references to Part II see page 151]. In this work attempts have been made to effect separations using a variety of methods, notably fractional crystallisation, fractional distillation, chromatography, and formation of urea complexes, none of which however gave entirely satisfactory results.

Assuming that a reasonably pure sample of an unsaturated acid is available the problem of determining its structure has still to be solved. Most analyses of unsaturated acids involve the rupture of the unsaturated linkages and the subsequent identification of the products. This method is quite satisfactory in the investigation of the mono-unsaturated acids, since there is no ambiguity in the interpretation of the results. However, in the case of the more highly unsaturated acids, complete oxidation yields products which can be characteristic of a number of different acids and consequently the constitution of these acids can be given only as a number of alternatives. (Tutiya 1941, Baudart 1943, cf. Klenk and Bongard 1952). It was therefore surmised that a process of incomplete oxidation of the unsaturated acids and analysis of the products might prove extremely useful: in this way the products of oxidation would give the positions of the double bonds without any ambiguity, e.g. if an octadecatetraenoic acid on examination

by this method gave a mixture of C_4, C_8, C_{12} and C_{15} dicarboxylic acids and a mixture of C_{14}, C_{10}, C_6 and C_3 monocarboxylic acids then the structure of the acid can be given as octadec-4:8:12:15-tetraenoic acid. However, it is obvious that a method such as this depends on the reliability of analysing the products of oxidation, which in this case were mono and dicarboxylic acids. Considerable time and attention was therefore paid to devising suitable means of estimating mono- and dicarboxylic acids.

Separation of Dicarboxylic Acids

As it is clearly desirable to be able to analyse very small quantities of material, a micro method was adopted. A technique which has been used in similar circumstances was that of partition chromatography as developed by Begemann, Keppler and Boekenoogen (1950). These authors used a column of silica gel to support an aqueous immobile phase consisting of a mixture of water, ethyl and methyl alcohols in fixed proportions. The mobile phase was formed of a benzene solution of the dicarboxylic acids under examination. About 20mg. of the dicarboxylic acids was dissolved in a few c.c. of the benzene phase and placed on the column of silica gel; the mixture was then developed by percolation of the benzene phase. The percolate was collected in 1 c.c. portions, all of which were separately titrated with a standard solution of alcoholic potassium hydroxide, thus giving an indication of the quantity of acid in the percolate. These authors further suggest

that by varying the column heights and liquid phases, the analysis can be adapted to any particular dicarboxylic acid or mixture of acids. The efficiency of this method was examined by constructing various columns under different conditions and using various solvents as the immobile phase. It was found that on using synthetic mixtures of dicarboxylic acids, it was difficult to obtain reproducible results using silica gel as the column carrier (prepared as Gordon, Martin and Synge 1943), and much better results could be obtained by using a diatomaceous earth (Celite 535) as the carrier. A similar material (Celite 545) has been used successfully by Peterson and Johnson (1948).

e.g. Test mixture 10 consisted of 6.4mg. dodecanedioic (C_{12}), 9.3mg. sebacic (C_{10}) and 16.4mg. azeleic (C_9) acid. This was dissolved in benzene, the solution placed on a column of Celite 535 and the analysis carried out (see experimental, p. 147) giving results as shown in Figure 4. A quantitative estimation of the amounts of the various acids was also made by determining the total quantity of standard alkali used to neutralise each acid. In this case the quantitative results are not very good (viz. 7,6mg. C_{12}, 11.9mg. C_{10} and 14.2mg. C_9) but this may be due to the inaccuracy of the blank measurements used. Nevertheless it

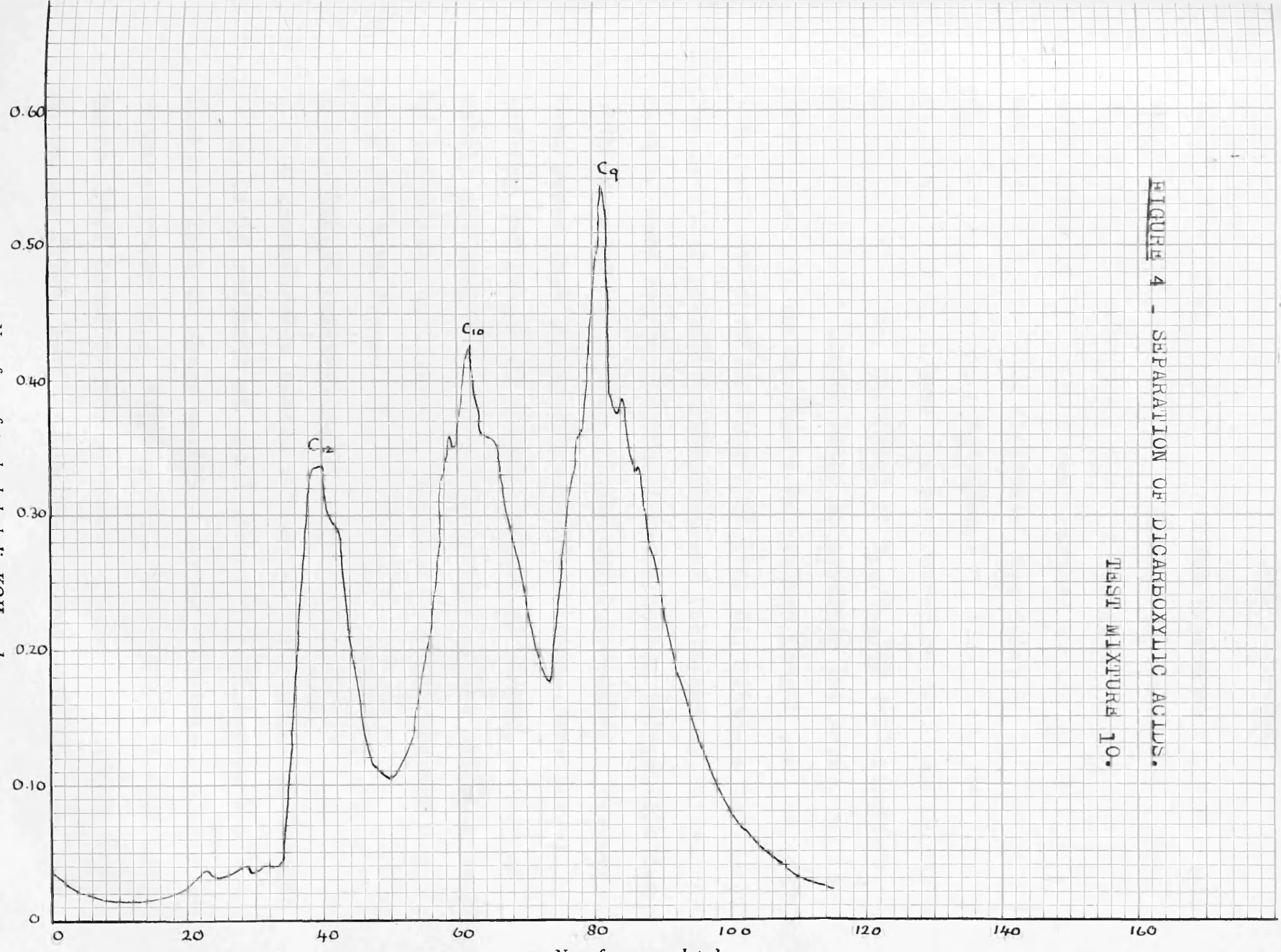


FIGURE 4 - SEPARATION OF DICARBOXYLIC ACIDS.

TEST MIXTURE 10.

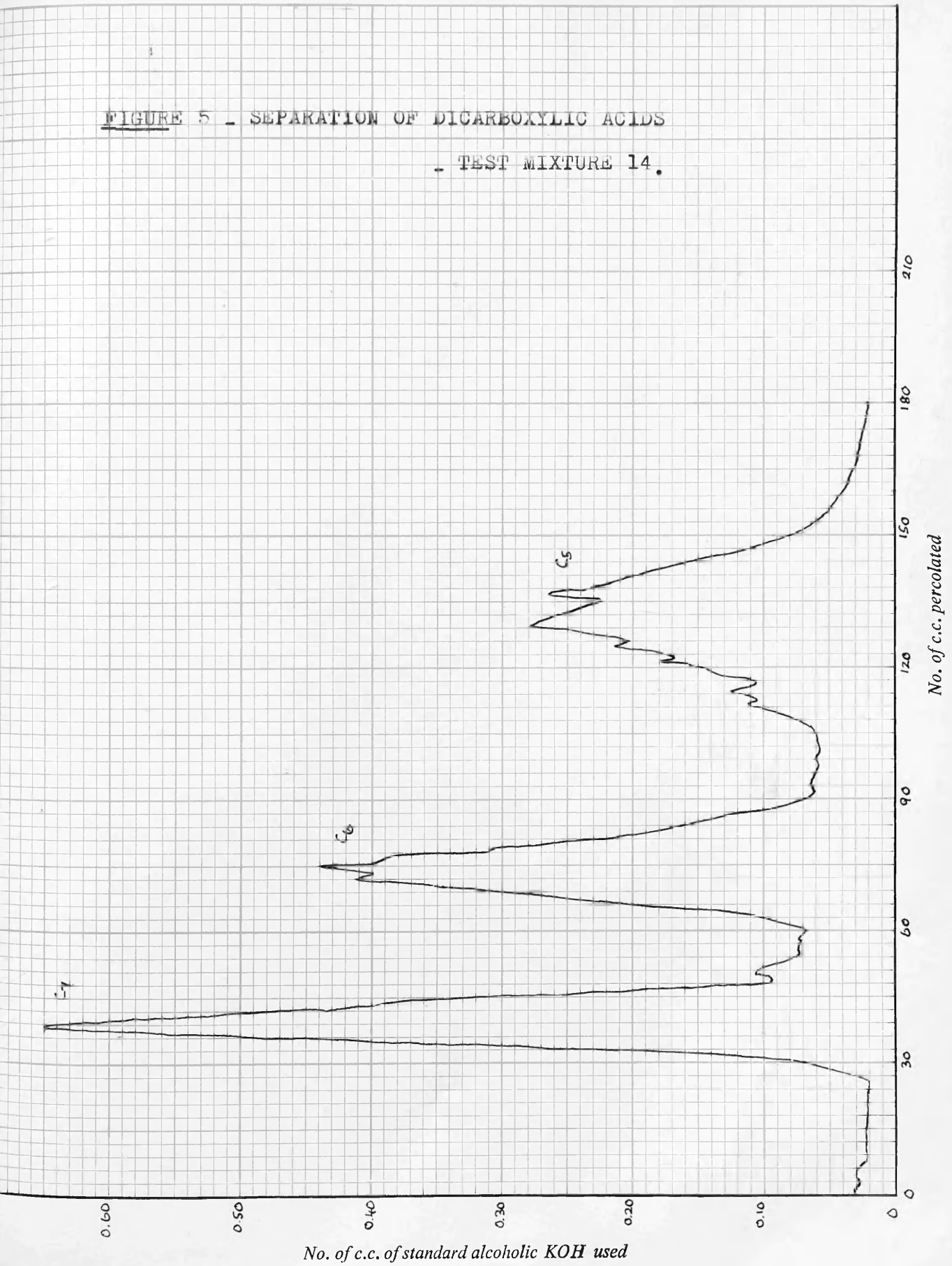
can be seen that a good qualitative separation has been achieved. Figure 5 shows a similar separation of 7.8mg. C_7, 7.1mg. C_6 and 7.4mg. C_5. A vigorous test of the efficiency of the method was provided by the separation of a mixture of 9 acids as follows:- C_{13} 4.2mg.; C_{12} 3.4mg.; C_{10} 3.4mg.; C_9 4.3mg.; C_8 5.3mg.; C_7 4.0mg.; C_6 4.6mg.; C_5 8.3mg. and C_4 2.6mg. A separation of these acids was achieved (with the exception of C_{13}/C_{12}) by employing two columns, one of length 34cm. to separate the higher acids giving results as in Figure 6A and one length 9cm. separating the lower acids as in Figure 6B (see experimental p.147). The identity of any particular peak (obtained by titration) with an acid can be confirmed by repeating the percolation with an authentic sample of the suspected acid. However it has been found that by keeping other conditions constant viz. packing and length of column, weight of sample, nature of mobile and immobile phases, the nature of the acid can usually by determined by observing the peak effluent volumes e.g. A, B and C in Figure 6B.

Separation of Monocarboxylic Acids

A considerable number of methods are available for the analysis of mixtures of small quantities of monocarboxylic acids. Most of these employ a system of partition chromatography (e.g. Nijkamp 1951, 1953, 1954, Marvel and Rands 1950) based on the procedure proposed by Ramsey and Patterson (1945, 1948). In this method the acids are

FIGURE 5 - SEPARATION OF DICARBOXYLIC ACIDS

- TEST MIXTURE 14.



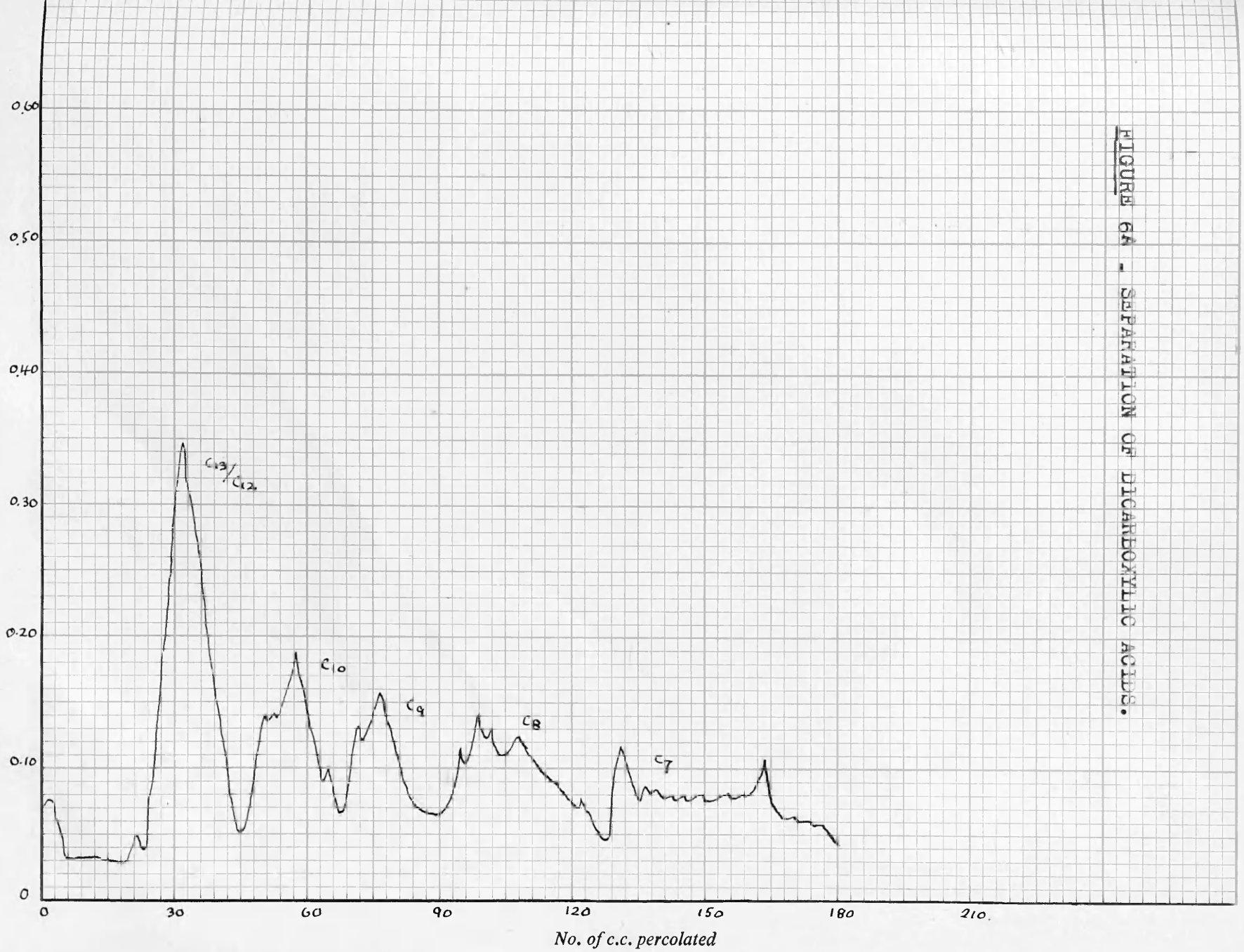


FIGURE 64 - SEPARATION OF DICARBOXYLIC ACIDS.

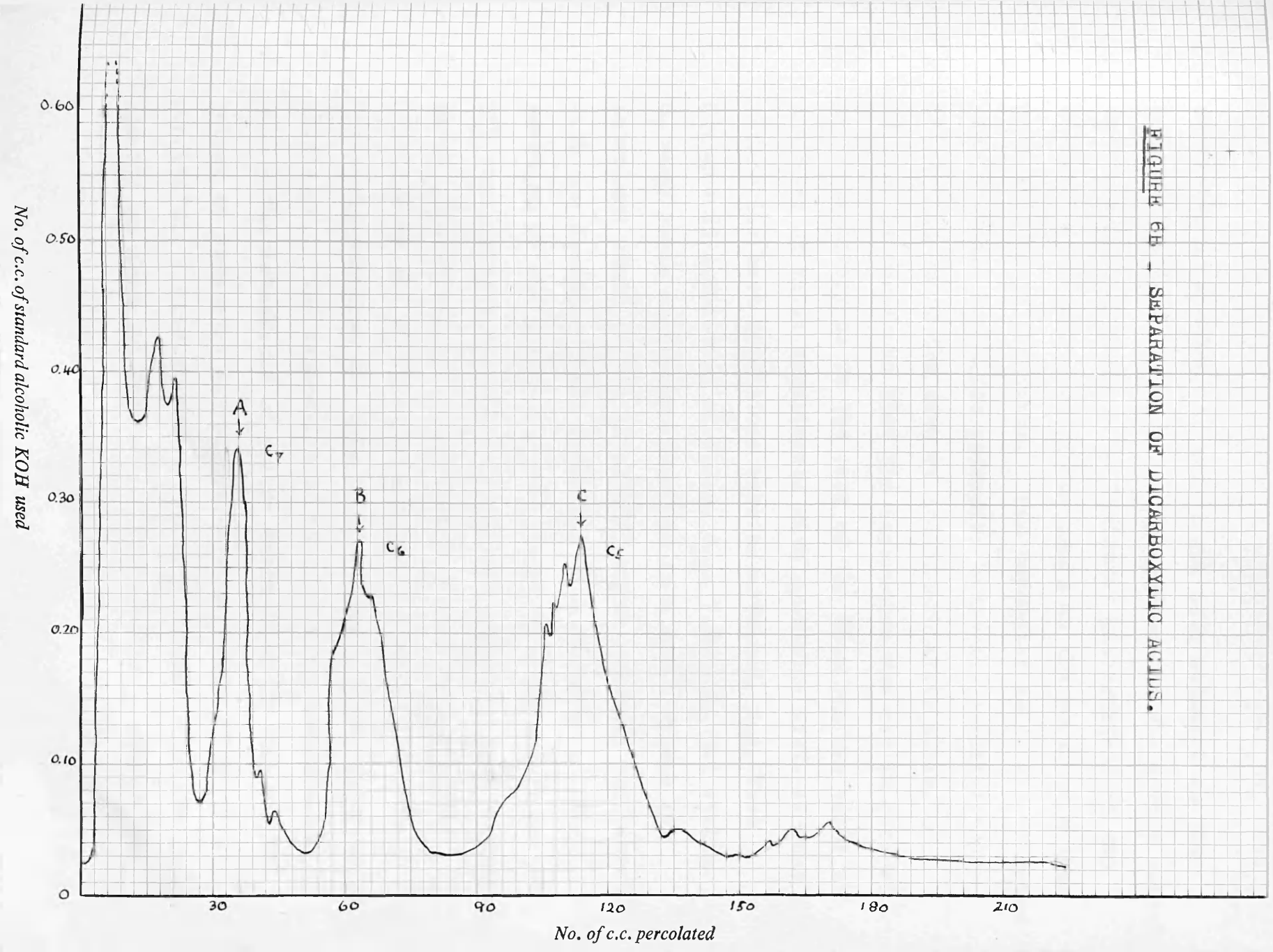
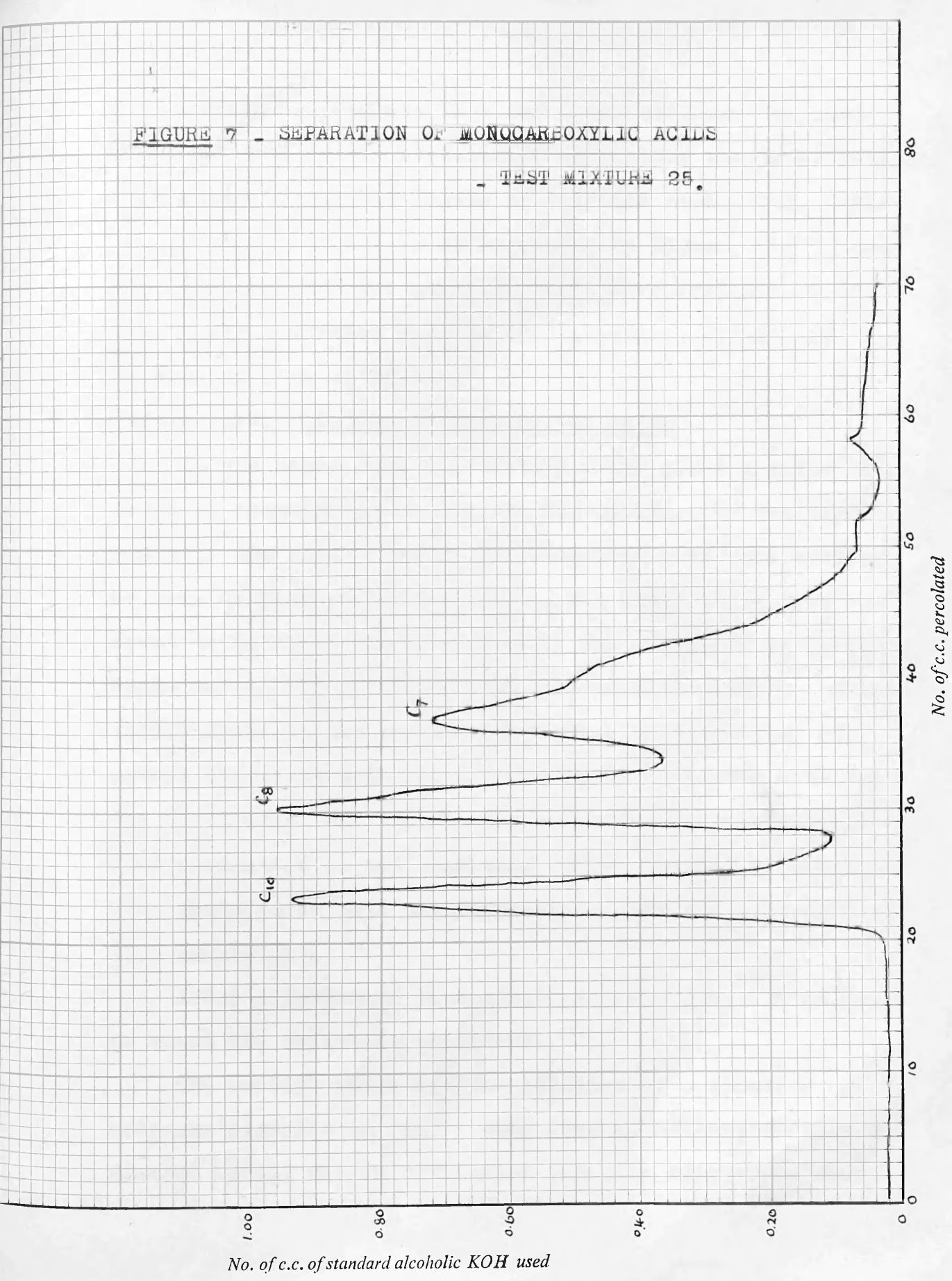


FIGURE 6B - SEPARATION OF DICARBOXYLIC ACIDS.

separated on a column of silica gel with methanol as the immobile solvent, 2:2:4 trimethyl pentane as the mobile solvent and bromocresol green as an indicator incorporated on the column. The positions of the acids on the column are thus indicated by yellow bands. The separated acids are titrated with standard alcoholic potassium hydroxide solution and identified as in the case of the dicarboxylic acids. However it was found that the separation was not in general as satisfactory as in the case of the dicarboxylic acids and a number of trial experiments with synthetic mixtures were made before the best conditions were ascertained. e.g. Test Mixture 25 consisting of 9.4mg. C_{10}, 7.5mg. C_8 and 11.1mg. C_7 mono basic acids was dissolved in a small volume of petroleum ether (b.pt. 120-150°C) which had been equilibrated with the immobile phase (methanol with 5% water). The solution of the acids was then placed on a column of Celite 535 containing the immobile phase and incorporating bromocresol green as indicator. The column had been made slightly alkaline by the addition of a few drops of $N/_{10}$ sodium hydroxide solution and this had imparted a blue colour to the indicator. The separation of the acids was ascertained by noting the detachment of yellow bands from the top of the column as each acid was eluted with petroleum ether (b.pt. 120-150°C). Titration with standard alcoholic potash gave results as shown in Figure 7. The identification of the

FIGURE 7 - SEPARATION OF MONOCARBOXYLIC ACIDS

- TEST MIXTURE 25.



acids could be presumed from the 'peak effluent' volumes and confirmed by the percolation of another sample of solution to which an authentic sample of acid had been added. The use of other indicators as suggested by Nijkamp (1953) was also investigated and although there were indications of improvement, further work on this aspect was abandoned because of lack of time.

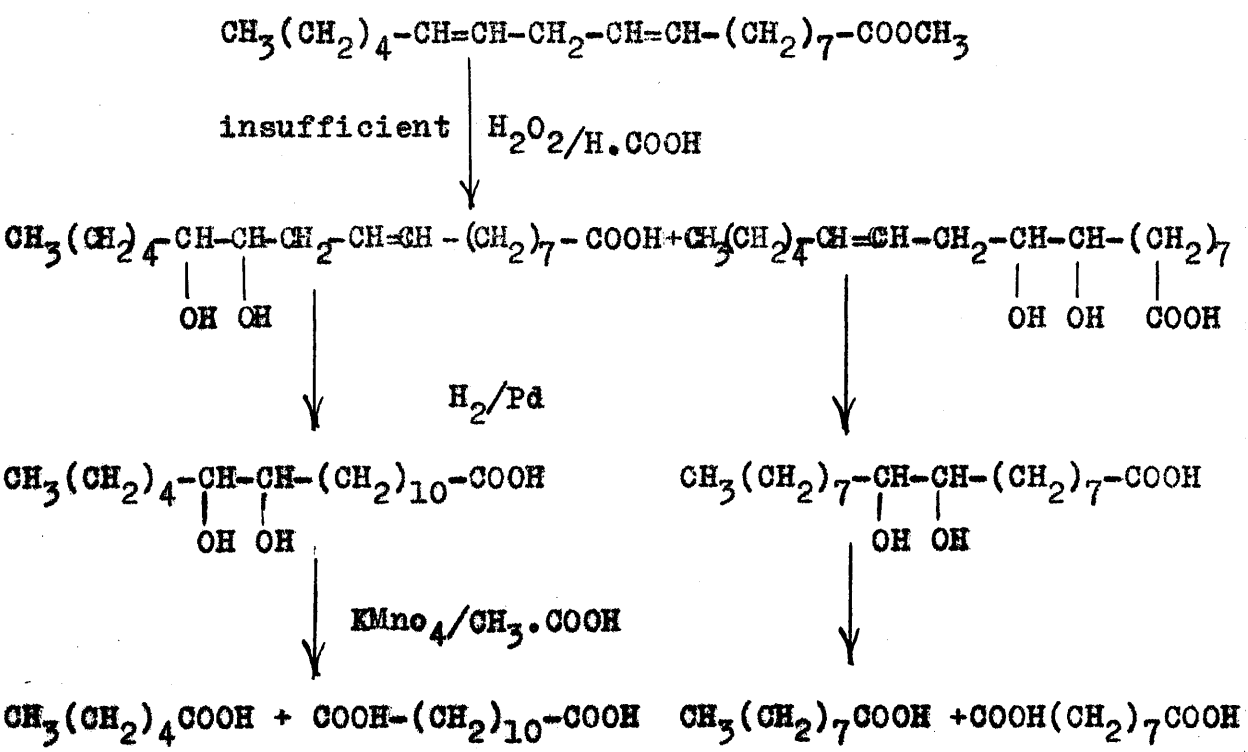
Partial Hydroxylation of Unsaturated Acids

It was hoped that this method would yield results which would be unambiguous as regards the position of the double bonds in the acid. Other investigators have used similar methods of partial hydrogenation, but these procedures were found to be ineffectual because of the migration of double bonds during the hydrogenation (see Hilditch 1949). Arimune (1951) found in the process of partial hydroxylation of methyl linoleate with hydrogen peroxide in acetic acid that the 12:13 ethylenic linkage was selectively hydroxylated. This contention is not confirmed by the present results. [The abstract of this paper appeared after the present results were completed].

After a number of preliminary experiments, it was decided to adopt the following procedure.

The initial stage was the addition of a solution of hydrogen peroxide in formic acid (Swern, Billen, Findley and Scanlan 1945), not in sufficient quantity however to cause

complete hydroxylation of all the double bonds in the molecule. Thus, for methyl linoleate 55% of the theoretical amount was used. This stage was followed by complete hydrogenation of the residual unsaturated centres. Oxidation of the resulting hydroxy acids with potassium permanganate in glacial acetic acid gave a mixture of mono-and dicarboxylic acids, which could be separated by steam distillation, the monocarboxylic acids being volatile. Analyses of these acids using partition chromatography then gave an indication of the positions of the ethylenic linkages in the original unsaturated acid. e.g. for methyl linoleate



The final stages of this procedure were first investigated by using 9:10 dihydroxystearic acid as the unknown acid; oxidation and identification of the resultant acids was successfully carried out. This technique was taken one stage further by using a mixture of 9:10 dihydroxystearic acid and 12:13 dihydroxystearic acid - the acids resulting on oxidation were also successfully identified. A much more rigorous trial of the method was carried out by partially hydroxylating a sample of methyl linoleate prepared by debromination of tetrabromostearic acid and purified by distillation. Analysis of the steam volatile material gave two peaks as shown in Figure 8, which were confirmed to be due to C_9 and C_6 monocarboxylic acids. However quantitative examination revealed that the hydroxylation had gone in very low yield (about 5%). Analysis of the dicarboxylic acids (see Figure 9) confirmed this by separation of the C_9 and C_{12} acids in a similarly low yield. Nevertheless these results implied that the double bonds in the original acid were in the 9 and 12 positions. Other investigators have also found that hydroxylations using performic acid do not give good yields (cf. Paul and Tchelitcheff 1954, McKay, Levitin and Jones 1954, Swern and Dickel 1954). A similar procedure was carried out on a sample of α-elaeostearic acid prepared from Nyasaland Tung oil, but the results were anamolous and it appears that this acid does not oxidise in the normal way (cf. Boëseken, Hoogland, Broek and Smit 1927).

FIGURE 8 - SEPARATION OF MONOCARBOXYLIC ACIDS FROM PARTIAL HYDROXYLATION OF METHYL LINOLEATE.

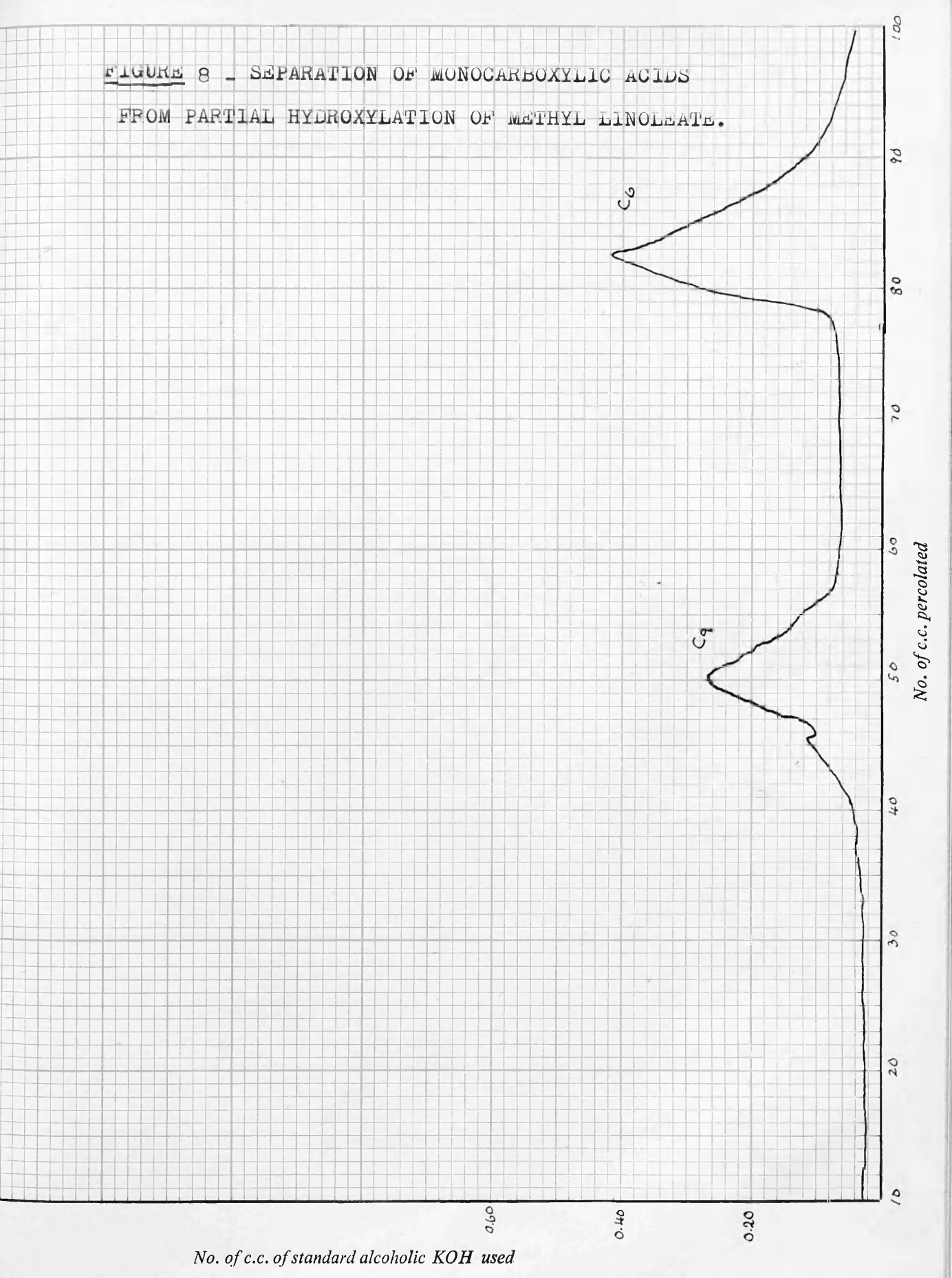
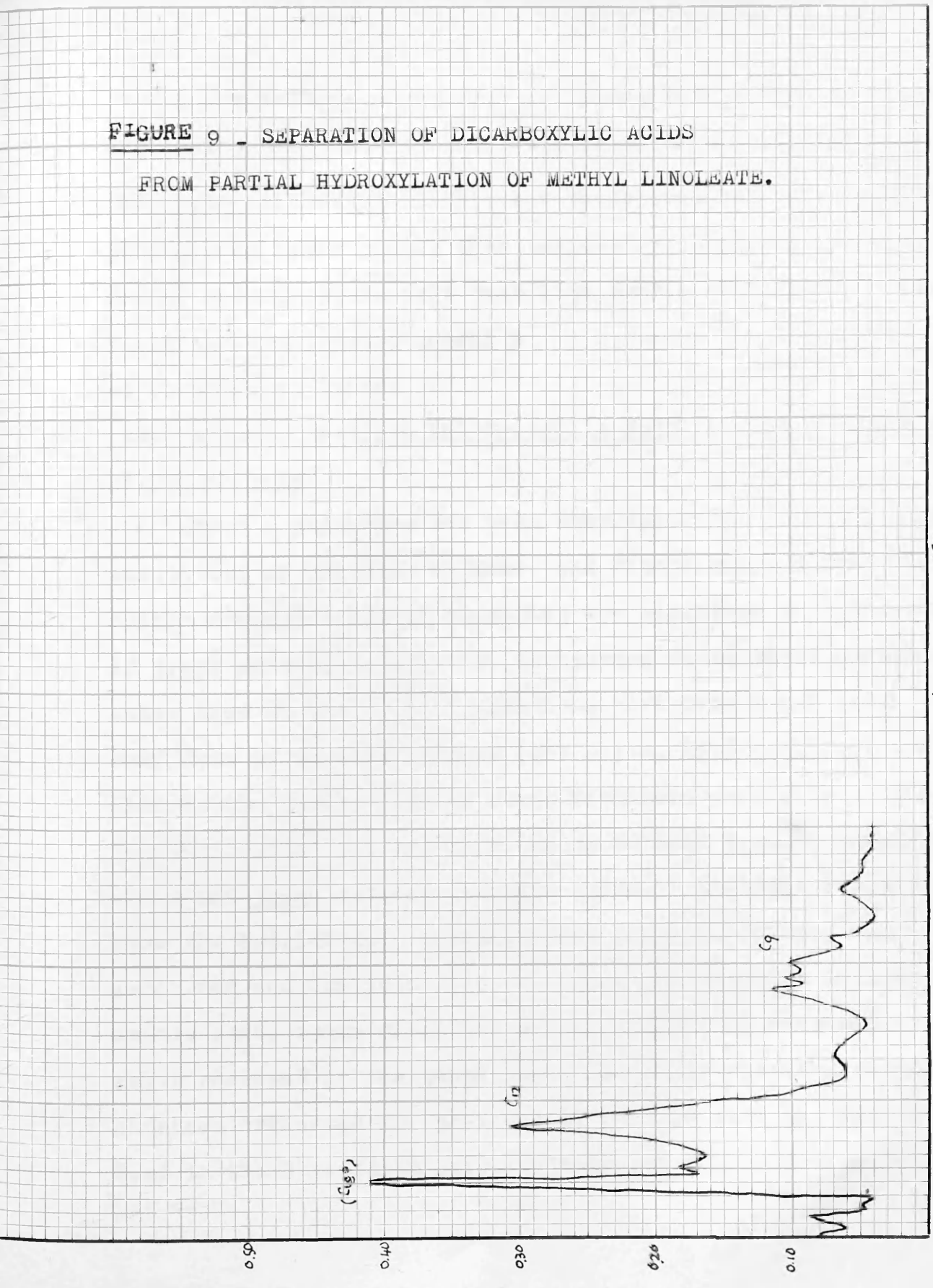


FIGURE 9 _ SEPARATION OF DICARBOXYLIC ACIDS

FROM PARTIAL HYDROXYLATION OF METHYL LINOLEATE.



Other methods of incomplete hydroxylation were attempted e.g. using potassium permanganate in alkaline solution (Lapworth and Mottram 1925), but these were not so successful giving even lower yields than the method involving performic acid. However it is felt that with further development this technique might prove useful in determining the structures of unsaturated acids.

Examination of Ostrich and Crocodile Fats for the presence of "Vaccenic Acid"

Since a procedure had been developed for analysing small quantities of mixtures of mono- and dicarboxylic acids, the opportunity was taken of examining the assertion that traces of trans octadecenoic acids, containing unsaturated centres other than in the $\Delta^{9:10}$ position, have been found in the body fats of various animals (see Gupta, Hilditch, Paul and Shrivastava 1950). These acids have been generally termed "vaccenic acid" and are reported to be both trans octadec-10-enoic and trans octadec-11-enoic acids. Suitable fractions from the ostrich and crocodile fats (in A fractions) were recovered and oxidised with potassium permanganate in glacial acetic acid, steam distilled and the resulting mixtures of acids analysed in the usual way. In every case the samples obtained indicated the presence of an acid with the double bond in the 9 position only. Infra red analysis also

showed the absence of any trans compounds (cf. antelope fat, Part I) and the identity of the acid with ordinary oleic acid. [The term "vaccenic acid" has been used rather loosely in several publications to denote mixtures of trans ethylenic acids. It is also worthy of note that Bumpus, Taylor and Strong (1950) have shown by X-ray diffraction methods that trans octadec-11-enoic acid is not identical with natural 'vaccenic' acid]

Re-examination of Crocodile Fat

All the previous work has presumed that a fairly pure sample of an unsaturated acid could be made available. It has already been indicated that this requirement is not easily met in the case of the complex mixtures usually associated with animal fats. As a large batch of crocodile oil (from C. niloticus) was available it was felt that further examination of the unsaturated acids of this oil could most readily be made. In the course of this examination methods of separating these unsaturated acids were investigated, as this was necessary before making any inquiries into their structures.

Separation of the Oil into Fractions by Distillation

About 1½ Kgm of the oil was methylated directly (Winter and Nunn 1950) in six batches, by refluxing in a solution of ether and methanolic potassium hydroxide. The esters were then submitted in samples of about 250gm. to fractional distillation under reduced pressure. After redistillation,

four main fractions were obtained by combination of fractions of similar boiling points, i.e.

Fraction A which consisted mainly of esters less than C_{16} in length.

Fraction B which contained mostly C_{16} esters.

Fraction C which contained mostly C_{18} esters.

Fraction D which contained mostly esters greater than C_{18} in length.

These four fractions were examined in greater detail:-

Fraction A

These esters (61.4gm.) were fractionally crystallised at -30°C. giving 41.2gm. insoluble material I.V. 4.3 and 18.1gm. of soluble material I.V. 66.2. The former fraction was submitted to fractional distillation and determination of the S.E.'s showed that myristic acid was the 'lowest' acid present. This acid was separated from one of the fractions giving a sample m.pt. 53-53.5°C. The soluble esters were also fractionally distilled, the S.E.'s indicating once more that there were no acids of chain length less than C_{14}. Suitable fractions were oxidised with alkaline potassium permanganate (Lapworth and Mottram 1925) to characterise the monoethenoid acids as dihydroxy acids. The crude dihydroxy acids were recrystallised from alcohol giving two batches of crystals, one of which on recrystallisation gave a small amount of a white crystalline material m.pt. 111.5-112°C.

[Found: C, 64.3; H, 10.5%. Calc. for dihydroxymyristic acid $C_{14}H_{28}O_4$: C, 64.6; H, 10.8%]. The second batch of crystals on recrystallisation gave another sample m.pt. 123°C. [Found: C, 64.9; H, 10.7%]. This suggests that there are two tetradecenoic acids present, the higher m.pt. derivative denoting the Δ^9 acid [cf. Boughton, Bowman and Ames, 1952], there being no indication in the literature of a dihydroxy myristic acid m.pt. 112°C. Although only a very small sample of this dihydroxy acid was available, oxidation with potassium permanganate in acetic acid was carried out. Separation of the products by steam distillation and partition chromatography suggested that the original tetradecenoic might have the double bond in the 7 position, although this was far from conclusive. If this supposition is proved to be correct it is evident that this acid will be analogous to oleic acid, in that the double bond is situated in the centre of the molecule.

Fraction B

Fractional crystallisation of this fraction at 0°C, -20°C. and -30°C resulted in the removal of about 180gm. of mostly saturated esters (probably methyl palmitate). A further crystallisation at -70°C gave two fractions viz. 102gm. of insoluble esters I.V. 84.8 and 28gm. of soluble esters I.V. 122.9.

The insoluble esters were fractionally distilled, and after determining the usual constants, suitable fractions were utilised to characterise the hexadecenoic acid which seemed to be the main component. Thus, a sample of esters (8.4gm., S.E., 268.0, I.V. 93.8) when submitted to isomerisation with a 7½% KOH/glycol solution at 180°C. for 60 min. showed an $E^{1\%}_{1cm}$ at 233mμ of 19.4 (i.e. about 2% of conjugated diethenoid acid present). A sample of the acids recovered from this fraction was oxidised with potassium permanganate in acetic acid and the products separated by steam distillation. Partition chromatography showed the presence of heptanoic and azeleic acids, the latter being also confirmed by the separation of a white solid m.pt. 100-103°C. (Mixed m.pt. with an authentic sample of azeleic acid was 103-105°C). The p-bromophenacyl ester of the acids derived from these ester samples was obtained as white crystalline needles m.pt. 39-39.5°C. [Found: C, 63.6; H, 7.8; Br, 17.7%. Calc. for p-bromophenacyl ester of hexadecenoic acid: C, 63.9; H, 7.8; Br, 17.7%. cf. m.pt. 39.5-40°C. for ester from synthetic hexadec-9-enoic acid (Baudart 1945)]. This work therefore confirms the presence of hexadec-9-enoic (palmitoleic) acid in crocodile oil.

The esters. soluble at -70°C. were also fractionally distilled, suitable fractions being recovered for further examination. The recovered acids (i.e. the most unsaturated) were isomerised in the usual manner giving results as follows:

$E^{1\%}_{1cm}$ at 234mμ = 154.9; $E^{1\%}_{1cm}$ at 269mμ = 37.7. Attempts were made to concentrate the polyethenoid esters in these fractions by separation on a column of silica gel and 'Celite' and eluting with petroleum ether (b.pt. 40-60°C.) [cf. Riemenschneider, Herb and Nichols 1949]. However this method did not yield satisfactory results. Separation of the C_{16} polyethenoid acids was then attempted by the preparation of the urea complexes. [cf. Silk, Sephton and Hahn 1954]. Although some concentration of the polyethenoid acids was achieved by this method, the presence of a complex mixture was still evident as is shown by the U.V. spectra after isomerisation viz. $E^{1\%}_{1cm}$ at 234mμ 521.1, at 269mμ 130.5 with weak maxima at 300 and 315mμ. A sample of these acids was brominated and it was found that a white solid appeared in the ether solution suggesting the presence of a hexabromide (cf. Markley 1947). This solid had a m.pt. 181-185°C and was soluble in hot benzene, a white precipitate m.pt. 189-192°C appearing on standing. Further recrystallisation gave m.pt. 190-192°C. (softens 188°C.) [Found: Br, 66.1%; Calc. for hexabromopalmitic acid $C_{16}H_{26}Br_6O_2$: Br, 65.7%]. A trace of material soluble in benzene but insoluble in petroleum ether (b.pt. 40-60°C.) m.pt. 99-102°C was obtained but there was insufficient for further investigation.

Fraction C

Esters from this fraction which were insoluble at 0°C.

(I.V. 28.7) were fractionally distilled. Recovery of acids from S.E. residues of appropriate fractions was followed by oxidation with alkaline potassium permanganate. On crystallisation from ethanol (after extraction of saturated acids with petroleum ether), the dihydroxy acids were obtained m.pt. 112-118°C. Further extensive recrystallisation was successful in yielding a sample m.pt. 125-126.5°C., although it was very evident from the difficulty of separation that the original dihydroxy acid was not homogeneous. A sample of this mixture of dihydroxy acids was oxidised with potassium permanganate in acetic acid in the usual way and the products analysed by partition chromatography. Although the results were far from conclusive the analysis of the monocarboxylic acids showed the presence of a $\Delta 9$ acid and possibly two others.

Further fractional crystallisation of this fraction at lower temperatures followed by distillation and urea complex formation merely confirmed the presence of linoleic acid by the separation of the tetrabromostearic acid m.pt. 109-110°C.

Fraction D

This fraction consisting mostly of esters of acids greater than C_{18} in length, was submitted to fractional crystallisation. However bromination of suitable fractions showed that complex mixtures were present e.g. Bromination gave an ether-insoluble white powder m.pt. 211-215°C. [Found, Br, 61.4%: Calc. for hexabromoarachidic acid

$C_{20}H_{34}Br_6O_2$: Br, 61.0%] and a small quantity of an ether soluble powder m.pt. 161-164°C. (tetrabromide?). There were no indications of monoethenoid acids in this fraction.

It will be seen that the results obtained from this series of experiments are rather unsatisfactory and further successful results await the introduction and development of new methods of analysing the complex mixtures so characteristic of this type of work.

Experimental

Partition Chromatography of Dicarboxylic Acids

It was found that for acids of C_7 or less a short column of length 9cm was sufficient to separate the acids. On this column any acids longer than C_7 appeared to percolate through in the first 20 c.c. without any appreciable separation. The column was prepared and the analysis carried out as follows:- 3gm. of 'Celite 535' was placed in a mortar and 3 c.c. of an aqueous phase consisting of 4 parts ethanol and 6 parts water were added and carefully distributed throughout the Celite by means of a pestle. About 25c.c. of the mobile phase (thiophene free benzene, an equal volume of which had been equilibrated with a volume of the immobile phase) was then added in small portions until a white slurry was obtained. This suspension was slowly poured into a glass column, 1cm. in diameter, having a small wad of cotton wool at the bottom constriction, and gently packed down with a perforated metal plunger, at the same time, making sure that the slurry never went dry. In this way a column 9cm. in length was obtained. The acids to be analysed were then dissolved in about 1c.c. of the equilibrated benzene and carefully added to the top of the column. Percolation was then carried out by adding small quantities of the equilibrated benzene to the top of the column. If the percolation appeared to be too slow it was accelerated by applying an overpressure with compressed

air. The percolate was collected in 1c.c. portions and titrated with standard alcoholic potassium hydroxide ($N/50$) using a microburette and thymol phthalein as indicator. (vigorous shaking was required near the end point).

For acids of length C_7-C_{12}, a longer column was required and this was obtained by slurrying 10gm. of Celite 535 with 10c.c. of an aqueous phase consisting of 3 parts ethanol, 4 parts methanol and 3 parts water. The analysis was then carried out as above.

Partition Chromatography of Monocarboxylic Acids

The column was prepared by placing 20gm. 'Celite 535' in a mortar and adding 10c.c. of the immobile phase (methanol with 5% water) containing 1c.c. of the standard indicator solution of bromocresol green. The aqueous phase was disseminated throughout the earth using the pestle, until the whole turned a pale yellow colour: 10 drops of $N/10$ methanolic potassium hydroxide solution were then added and the Celite again mixed with the pestle until a uniform deep blue colour was obtained. About 50c.c. of petroleum ether (b.pt. 120°-150°C.) (mobile phase) was then slowly added to the Celite until a slurry was produced. This suspension was added to the column and the packing and the subsequent analysis carried out as in the case of the dicarboxylic acids. However, in this case the course of the separation could be followed visually by the separation of yellow bands against a blue background.

Partial Hydroxylation of Methyl Linoleate

1.00gm. of methyl linoleate was mixed with 3c.c. of formic acid (98-100%) and 0.36c.c. of hydrogen peroxide solution added [i.e. enough to react with 1.05 double bonds; strength of the peroxide was checked and found to be 33.5gm. hydrogen peroxide per 100c.c. of solution]. This suspension was shaken at room temperature for about 1 hour and then saponified by adding 30c.c. of 5N alcoholic potassium hydroxide and refluxing on the water bath for about ½ hour. The resulting soap solution was then acidified by the addition of 15c.c. of 5N HCl, cooled and extracted with about 75c.c. chloroform (3 x 25c.c.). This solution was dried with anhydrous sodium sulphate and the solvent removed giving 0.93gm. material, which was dissolved in alcohol and hydrogenated using palladium charcoal as catalyst. When no more hydrogen was absorbed the catalyst was filtered off, some of the alcohol removed by distillation, water added and the resultant dihydroxy acids extracted with chloroform (3 x 25c.c.) and the solution dried with anhydrous sodium sulphate. Removal of the solvent gave 0.73gm. of solid oxidised by dissolving in 21.8c.c. of glacial acetic acid and adding 1.82gm. of powdered potassium permanganate. This solution was then shaken at 50°C. for 4 hours. Most of the acetic acid was then distilled off by heating on the water bath at 40°C. under reduced pressure. About 30c.c. of water was added and sulphur

dioxide passed through the solution until there was complete decolorisation. The solution was then steam distilled until about 200c.c. of distillate was collected. This distillate and the residue from the steam distillation were both extracted with chloroform and the solutions dried with anhydrous sodium sulphate. Removal of the solvent gave 63.4mg. of steam volatile material and 254.9mg. of residue. The steam volatile material was then dissolved in equilibrated petroleum ether (b.pt. 120-150°C.) (see page 149) and made up to 5c.c. in a standard flask. One c.c. of this solution was withdrawn and examined by partition chromatography for mono-carboxylic acids, showing the presence of C_9 and C_6 acids. [Results showed about 31% C_6 and 33% C_9 - remainder assumed due to formic and acetic acids]. The residue from the steam distillation was extracted with hot petroleum ether (b.pt. 60-80°C.). On allowing to cool, the supernatant liquid containing saturated acids was decanted off and the residual dicarboxylic acids heated on the steam bath to dryness (115.9mg) These acids were dissolved in equilibrated benzene and made up to 5c.c. in a graduated flask. One c.c. of this solution was withdrawn by pipette and analysed for dicarboxylic acids in the usual manner, showing the presence of C_9 and C_{12} acids. This procedure thus confirmed the structure of linoleic acid as octadeca-9:12-dienoic acid.

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Part III - The Constitution and Properties of Santalbic Acid

Introduction

Analyses of vegetable fat composition have shown that, in contrast to the animal fats, the component acids of seed fats are often characteristic of the families into which plants have been divided from consideration of morphology and physiology. The following account deals with an investigation of the constitution and properties of an acetylenic acid, a type of acid that has not yet been found in animal fats (cf. page 72).

Sandalwood is a highly scented wood, and as such finds extensive use in the many Buddhist temples throughout India. The tree from which the wood is obtained (Santalum album. Linn.) is an evergreen and there are considerable plantations in all parts of India; it bears fruit twice a year and its seed has been found to be rich in oil.

The oil from the seed of Santalum album L. has been the subject of several preliminary investigations. (Rao et. al. 1934; Rao 1937; Iyer 1935; Sreenivasaya and Narayana 1928, 1936; Kotasthane and Narayana 1938). [For references to Part III see page 182]. These workers have shown that the seeds contain an oil which readily polymerises, and some of them suggested that the oil might be commercially important. The first detailed examination of the oils was undertaken by Madhuranath and Manjunath (1938). These investigators found that the oil on hydrolysis consisted essentially of one component and a trace of palmitic, oleic and linolenic acids.

This major component was a solid acid m.pt. 41-42°C., for which analysis indicated the formula $C_{18}H_{30}O_2$. On hydrogenation 3 moles of hydrogen were absorbed giving stearic acid. The acid had an I.V. of 133 and they made an unsuccessful attempt to prepare a maleic anhydride adduct. The acid was found to be different from punicic acid m.pt. 44°, a stereoisomer of elaeostearic acid, and on this evidence Madhuranath and Manjunath postulated that the acid was an octadecatrienoic acid, which appeared to be non-conjugated and named it santalbic acid.

Santalbic acid: Isolation and constitution

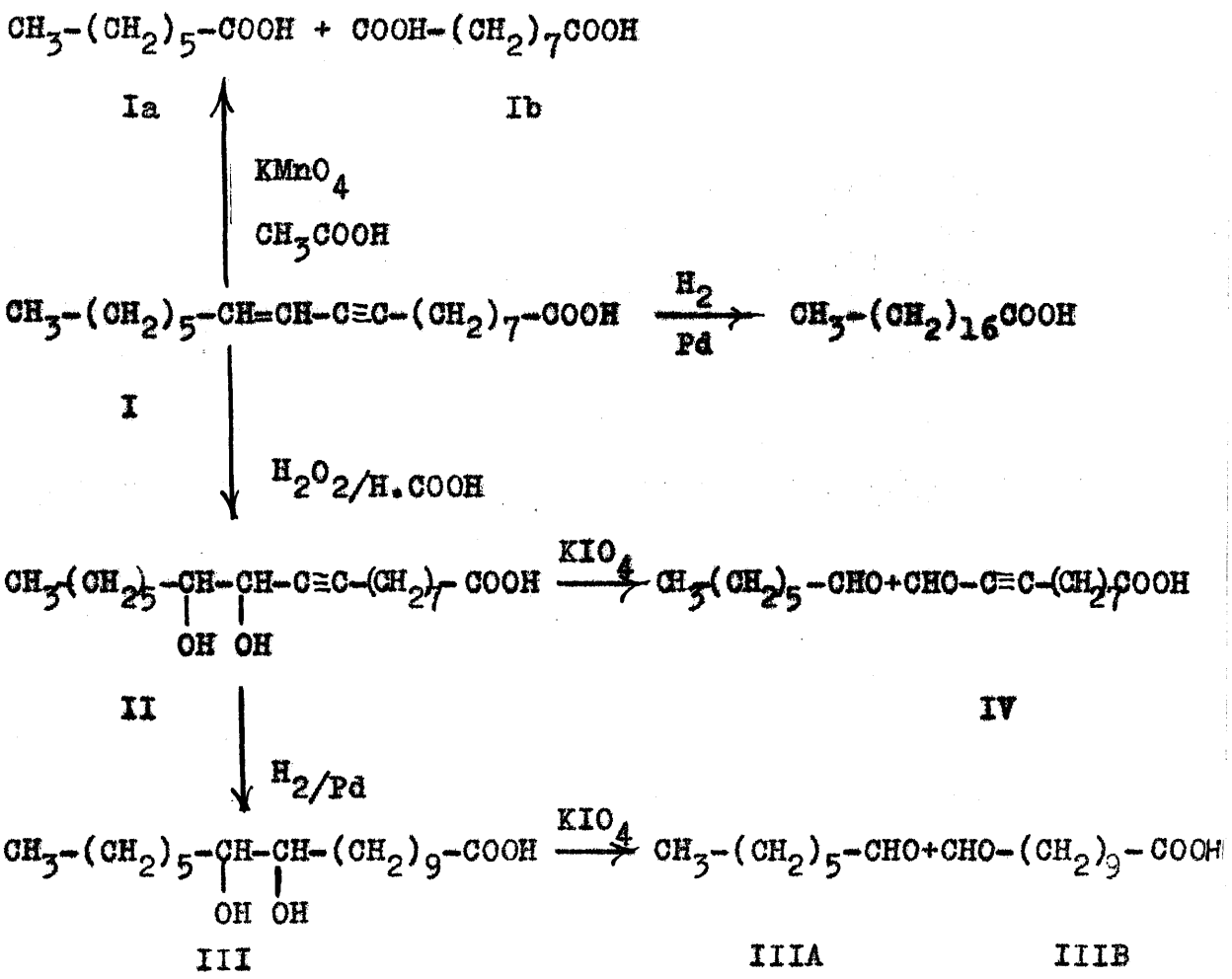
A sample of the seeds of Santalum album L. was obtained in this laboratory and no difficulty was found in extracting santalbic acid.

The dried seeds were brown in colour and in size were similar to small peas. The seeds when crushed in a mortar and extracted with light petroleum yielded a pale green viscous oil (53.5% of weight of seeds), which on hydrolysis gave the mixed acids as a pale yellow oil. During hydrolysis a rubbery substance separated out; this material seems to be characteristic of the oil (e.g. Madhuranath and Manjunath found 5.2% present in the oil; in this case an estimation of the amount of unsaponifiable material gives the figure of 6.9%). Crystallisation of the mixed acids from light petroleum gave crude santalbic acid (ca. 75%: m.pt. 36-38°C.) readily purified by repeated crystallisation (66%; m.pt. 38.5-39.5°C).

In view of the possible commercial importance of the seeds, a sample of santalbic acid was obtained directly from the seeds. The crushed seeds were hydrolysed with alcoholic potassium hydroxide i.e. without any preliminary extraction of the oil. On acidification and extraction with ether, the mixed acids were obtained and santalbic acid extracted in a yield of 19.3% (of weight of seeds).

An ultraviolet absorption examination of santalbic acid showed a maximum at 229mμ (log ε 4.22) and a point of inflexion at 240mμ (log ε 4.06). These values indicated that the acid did not contain a conjugated triene as chromophore, but probably an enyne system (Heilbron, Jones and Weedon 1944). A naturally occurring enyne acid has been described (Lighthelm, Schwarz and von Holdt, 1952; Ahlers and Ligthelm 1952) and a comparison of the two acids and their p-bromphenacyl esters revealed their close similarity. The identity of this acid, ximenynic acid, with santalbic acid has now been confirmed by hydrogenation, oxidation and infra red spectrum (preliminary note: Gunstone and McGee 1954) and by comparison with a synthetic sample (Grigor, McInnes and McLean, 1954, 1955).

Chemical evidence for the structure of santalbic acid has been shown by the following series of reactions.



Santalbic acid (I) readily absorbed 3 moles of hydrogen giving stearic acid and oxidation with potassium permanganate in acetic acid (Begemann, Keppler and Boekenoogen 1950) yielded n-heptanoic acid (Ia) and azeleic acid (Ib). These facts combined with the U.V. spectra data showed that the acid had one of the following structures.

A) $CH_3-(CH_2)_5-CH=CH-C\equiv C-(CH_2)_7-COOH$

B) $CH_3-(CH_2)_5-C\equiv C-CH=CH-(CH_2)_7-COOH$.

The position and configuration of the double bond was characterised by utilising the action of performic acid

(Swern, Billen, Findley and Scanlan 1945). A triple bond has been shown to be resistant to the action of peracids (Malenok and Sologub 1936, 1940, 1941; Malenok 1939) and consequently the en-yne system of santalbic acid will be oxidised preferentially at the double bond to give 11:12 dihydroxystearolic acid (II). On catalytic hydrogenation of (II) an 11:12 dihydroxystearic acid (III) was obtained m.pt. 126-126.5°C. [a later sample on crystallisation gave m.pt. 127-128°C.]. The structure of this acid was shown by oxidation with potassium periodate (cf. Gunstone 1954), the products being heptanal (IIIA) and 10-formyldecanoic acid (IIIB) which was subject to further oxidation with potassium permanganate to give 1:11 undecanedioic acid.

Previous work on the 11:12 dihydroxystearic acids (Bounds, Linstead and Weedon 1954) shows that the erythro acid has a m.pt. about 129°C. (values from 125.5-126°C. to 129-130°C. have been reported) and the threo acid about 94°C. It is therefore evident that III must be erythro 11:12 dihydroxystearic acid, and since performic acid gives trans addition to the double bond, it follows that the original double bond of the saltalbic acid must have had the trans. configuration. (cf. Raphael 1949). The trans nature of the double bond has also been confirmed by infra red spectroscopy (Gunstone and McGee 1954). From the foregoing evidence, santalbic acid must have the structure A) with the double bond having the trans configuration.

Further Reactions on Santalbic Acid and its Derivatives

The structure of 11:12 dihydroxy stearolic acid (II) was confirmed by oxidation with potassium periodate (cf. Gunstone 1954) to give heptanal and 10-formyldec-9-ynoic acid (IV). Although not very stable this compound was satisfactorily analysed, its ultraviolet absorption measured and a 2:4 dinitrophenylhydrazone prepared. This latter compound is a deep yellow colour in contrast to the orange and red colours generally apparent in the 2:4 dinitrophenylhydrazones of αβ unsaturated aldehydes and ketones (e.g. 2:4 dinitrophenylhydrazone of 11-formylundec-10-enoic acid was obtained from ethanol as deep orange crystals: Gunstone 1954). Similar yellow 2:4 dinitrophenylhydrazones have been reported from but-2-ynal and hept-2-ynal (Lunt and Sondheimer 1950). This decreased colour intensity is probably due to the fact that the Υ electrons of a triple bond are more tightly bound than those of a similarly situated double bond (Raphael 1955a). The ultra-violet spectrum of this acetylenic aldehyde (IV) is interesting in that a higher intensity of absorption was observed in hexane solution than in a solution of rectified spirits; and in addition other maxima were noted in the former solvent which have previously not been reported (see Figure 10). The lower absorption in rectified spirits may be due to hemiacetal formation as suggested by Ashdown and Kletz 1948 (cf. Crombie 1955a).

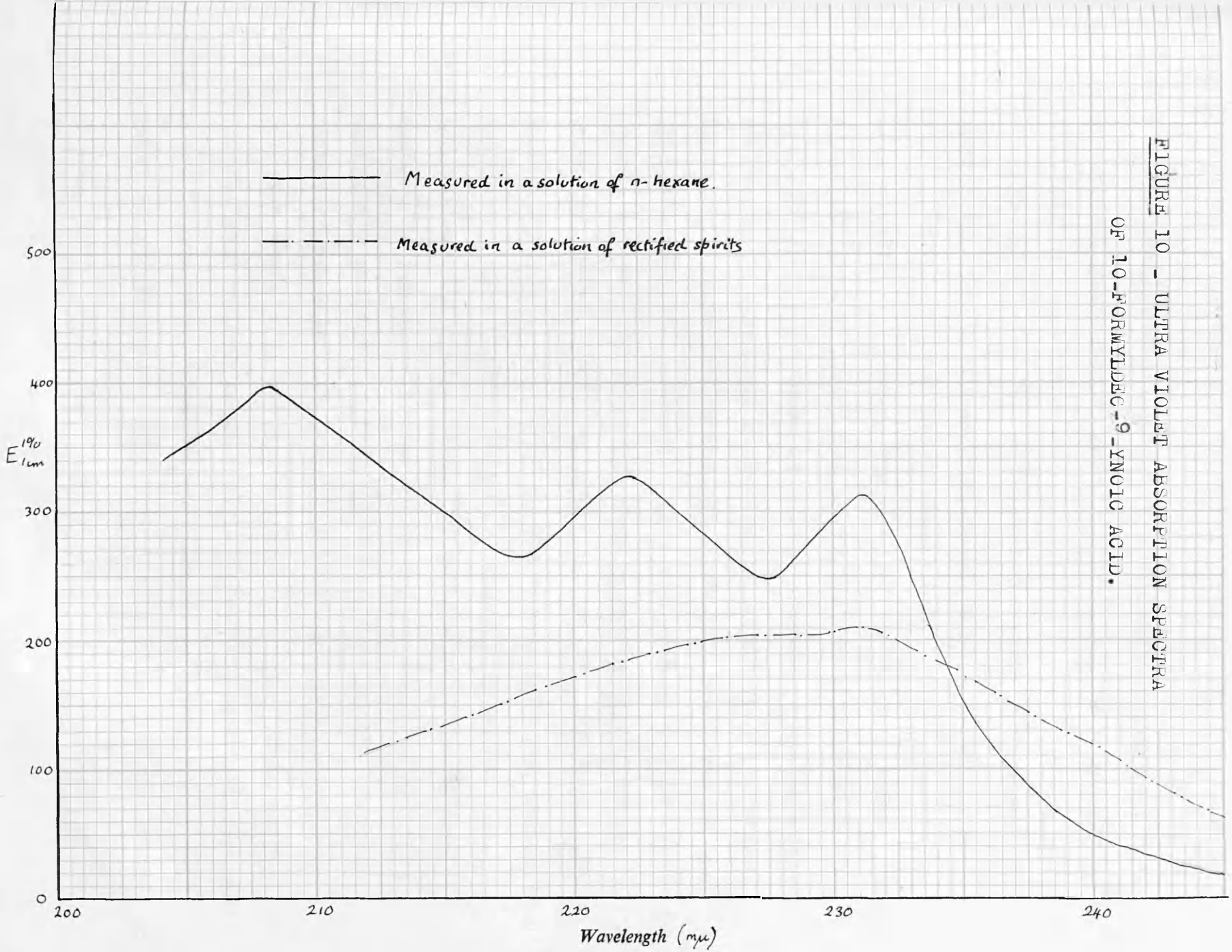


FIGURE 10 - ULTRA VIOLET ABSORPTION SPECTRA OF 10-FORMYLDEC-9-YNOIC ACID.

Dihydroxystearic acid (III) was further oxidised with potassium permanganate in acetic acid to give heptanoic acid and 1:11 undecanedioic acid.

Previous attempts to convert the enyne system of ximenynic acid to the corresponding conjugated diene by partial hydrogenation using a poisoned catalyst proved unsuccessful. (Ligthelm, Schwartz and von Holdt 1952). However, Lindlar (1952) has shown that on shaking with hydrogen in the presence of a lead poisoned palladium-calcium carbonate catalyst and quinoline, a triple bond is reduced to a double bond having the cis configuration (even in the presence of double bonds). Thus methyl deca trans-2-en-4-ynoate was reduced to methyl deca trans-2-cis-4-dienoate (Crombie 1955b). This catalyst has also proved invaluable in the syntheses of vitamin A, the carotenoids and analogous substances (See Raphael 1955d). Using this method, a conjugated acid m.pt. about 0°C. was obtained from santalbic acid. In ethanol solution there was no break in hydrogen absorption and the reaction was stopped after the required amount of hydrogen had been absorbed; in light petroleum solution there appeared to be no absorption. This conjugated dienoic acid on examination by ultra-violet spectroscopy gave a broad band with a maximum at 231mμ (ϵ 24,000) and a weak point of inflexion at 240mμ (see Figure 11). (This latter

characteristic might of course be due to traces of santalbic acid in the product, since this acid was not purified). These results agree with the properties of octadeca-*cis*-9-*trans*-11-dienoic acid as prepared by Nichols, Herb and Riemenschneider (1951) from alkali isomerised linoleic acid viz. m.pt. -6° to + 3°C. and maximum at 233mμ (ϵ 24,400).

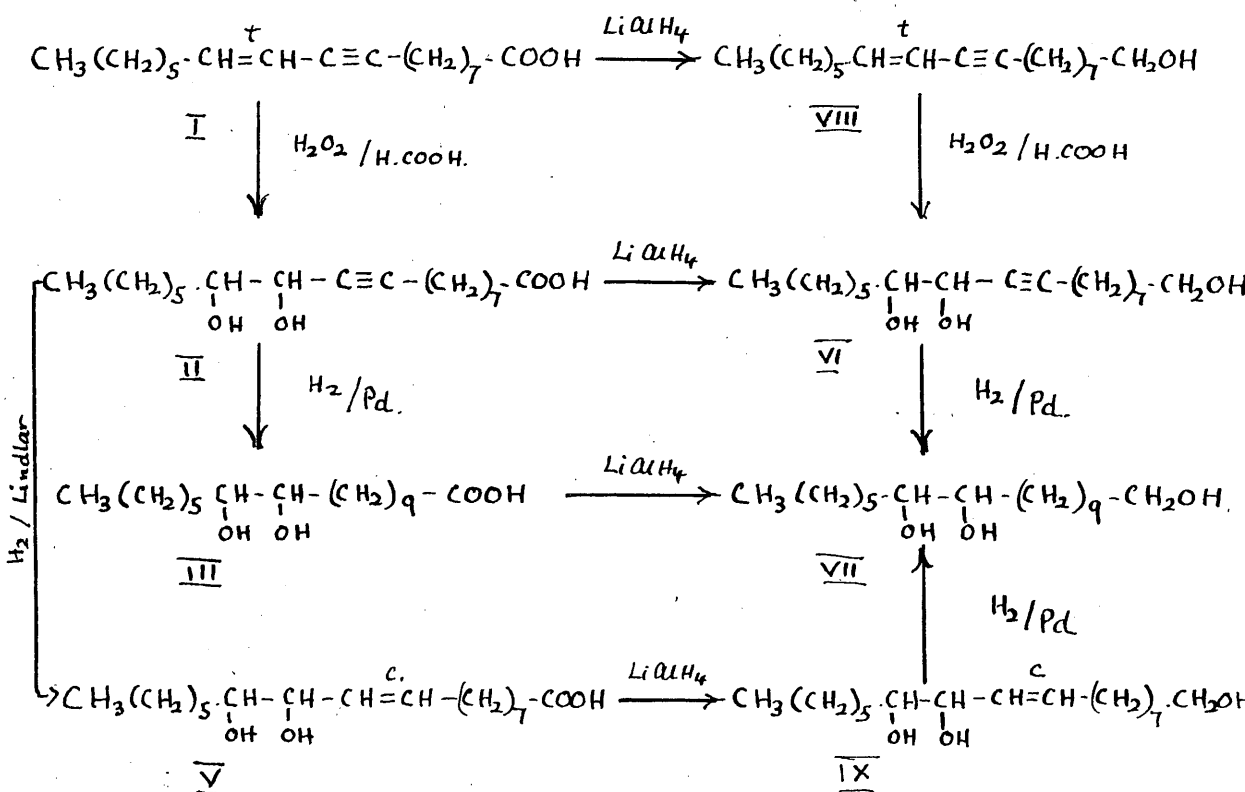
In the presence of iodine this acid readily isomerised (Nichols, Herb and Riemenschneider 1951) to produce the corresponding *trans*-*trans* acid m.pt. 53-54°C., which showed ultraviolet absorption spectra with a maximum and two points of inflexion (see Figure 11). The maximum at 231mμ (ϵ 33,300) and points of inflexion at 227mμ and 239mμ confirm the general observation that the *trans* isomer shows more structure in the region of maximum absorption than the corresponding *cis* isomers (cf. maximum at 231mμ with ϵ 32,200 obtained by Nichols *et al*. 1951). It is interesting to compare the ultra-violet absorption spectrum of the *trans*-*trans* acid (Mangold's acid: Mangold 1894) with the proposition that a conjugated diene should exhibit three cusps, and that the failure to find these cusps is in general due to the lack of sufficiently good resolution in spectrophotometers (Hammond and Lundberg 1953). Kass (1944) has also published an absorption spectrum for this acid (obtained from dehydrated castor oil), showing a maximum at 232mμ (ϵ 32,200) and two points of inflexion at 224mμ and 239mμ [Van der Hulst(1935) reports a maximum at 231mμ (ϵ 33,600) for this acid].

FIGURE 11 - ULTRA VIOLET ABSORPTION SPECTRA OF OCTADECA-9:11-DIENOIC ACIDS.

Evidence for the trans-trans conjugated nature of Mangold's acid was further confirmed by the preparation of the maleic anhydride adduct.

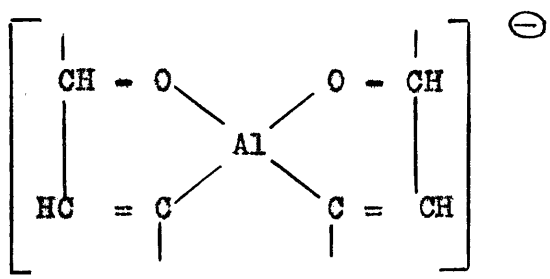
Another reaction in which use was made of Lindlar's catalyst was the preparation of 11:12 dihydroxyoctadec-cis-9-enoic acid (V) (i.e. 11:12 dihydroxyoleic acid).

Selective hydrogenation of a triple bond can also be carried out using lithium aluminium hydride. It has been shown that this reagent furnishes excellent yields of trans ethylenes from acetylenes if the triple bond is flanked by a propargylic hydroxyl group. (Raphael 1955b, Bates, Jones and Whiting 1954).



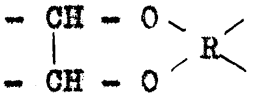
11:12 Dihydroxystearolic acid (II) seemed to have the structure necessary for the reduction of the triple bond and attempts were made to reduce this acid with the reagent to the corresponding trans-ene triol. This reaction was carried out several times using increasing quantities of lithium aluminium hydride and longer reaction times, but the only product obtained was the yne-triol (VI) The structure of VI was confirmed by preparing ximenynyl alcohol (VIII) (Ligthelm, Rudloff and Sutton 1950) and subsequently oxidising this with performic acid to 1:11:12 trihydroxyoctadec-9-yne (VI).

The selective reduction with lithium aluminium hydride has been explained (Attenburrow et al 1952) by the probable formation of an intermediate aluminium complex of the type:-

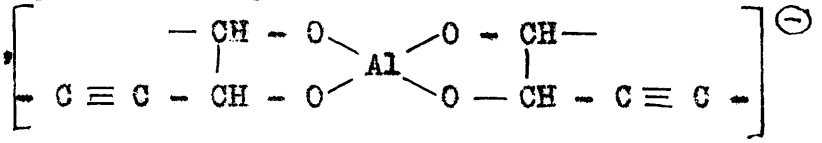


This mechanism accounts for the necessary presence of a propargylic -OH group and for the trans configuration of the product. The only other exception to this selectivity has been the case of a compound in which a bulky trimethylcyclohexenyl group was α to the triple bond. Steric hindrance probably prevents the formation of a complex of the above

type (Raphael 1955c). It therefore seems that there is a further limitation of this reaction in that it is not applicable to acetylenic glycols of the type -C(OH).C(OH)-C≡C-. It is well known that the vic glycols easily form metal complexes; thus the oxidative cleavages involving 1:2 glycols and formation of aldehydes have been shown to be dependent on the initial formation of complexes of the type



(Heidt, Gladding and Purves 1945). It therefore seems likely that the preferential formation of a complex of the type,



in the case of 11:12 dihydroxystearolic acid hinders the formation of the complex involving the transition of the triple to the trans double bond.

Catalytic reduction of the -yne triol (VI) gave 1:11:12 trihydroxyoctadecane (VII), also obtained by lithium aluminium hydride reduction of the 11:12 dihydroxystearic acid (III) and by catalytic reduction of the 1:11:12 trihydroxyoctadec-cis-9-ene (IX), the latter cis ene triol being obtained by lithium aluminium hydride reduction of the dihydroxyoleic acid (V).

Santalum album Seed Oil. From spectroscopic data, it is evident that the mixed acids (excluding unsaponifiable material) contain 95% of santalbic acid along with 5% of other

unidentified acids. The ultra-violet absorption of the oil itself gives a content of 88% santalbic acid (as glycerides). From this U.V. data then the approximate composition of the oil can be given as 88% santalbic acid 7% unsaponifiable material and 5% minor acids.

The en-ynoic acid has now been recognised by Ligthelm, Horn, Schwartz and von Holdt (1954) in three South African Ximenia species (Natural order; Olacaceae): X. caffra, X. caffra var. natalensis, X. americana var. microphylla, and by Hatt and Szumer (1954) in two species of the Santalum genus (Natural Order; Santalaceae): S. acuminatus (D.C.) (the sweet Quandong) and S. Murrayana (F.v. M) (the bitter Quandong). The contents of oil and proportions of ximenynyl glyceride in these oils are compared with the present sample in Table 60.

Table 60

Oil	% oil extracted	% ximenynyl glyceride
S. acuminatus D.C.	55-60	40-43
S. Murrayana F.v.M	65-70	
Ximenia oils	64.1-68.4	22-24
S. album L.	53.5	88

There has been no evidence for the presence of a hydroxy acid in the oil from S. album L. as has been found in the X. caffra

seed oil (Ligthelm 1954). It is apparent from the figures in Table 60 that sandal seeds provide the best source of this enynoic acid, being readily isolated in high yield by crystallisation of the mixed acids. It is interesting to note that the Ximenia and Santalum genera belong to closely related families grouped in the santalales and apart from two analyses (Puntambeker and Krishna 1937; Boekencogen 1939) of X. Americana seed oil, which may be in error (cf. Ligthelm et al. 1954) each species of these two genera yet examined contains this enynoic acid. Hatt and Szumer (1954) in their examination of the two Australianmembers of the Santalaceae, suggested that santalbic acid from S. album would prove identical with ximenynic acid. As a full examination of oils from other species of Santalum is envisaged by these workers it will be interesting to see if this acid provides another illustration of a particular acid being characteristic of a botanical classification. (An account of this work has been accepted by the Chemical Society for publication in the Journal).

Experimental

Absorption spectra were determined with a Unicam quartz spectrophotometer using rectified spirits as solvent (except where otherwise stated). Light petroleum refers to the fraction of b.pt. 40-60°C.

Santalum album L. Seed Oil:

The dried seeds (100 = 15.11gm.) were coarsely ground in a mortar and extracted (Soxhlet) with light petroleum. Removal of the solvent left a viscous pale greenish-yellow liquid of I.V. 152.5 and S.E. 332.8.

Isolation of Santalbic acid:

The oil (131gm.) was hydrolysed by refluxing with alcoholic potash (53gm. KOH/53gm. H_2O in 780c.c. alcohol) for one hour. During the hydrolysis, a gummy material separated out, which was rejected in the later extraction. About 400c.c. of alcohol was distilled from the hydrolysate, and extraction of the acids carried out with ether after acidifying the soaps with 25% sulphuric acid and adding plenty of water. On drying the ethereal solution with anhydrous magnesium sulphate and removing the solvent, 103gm. of a golden yellow oil was produced. Crude santalbic acid (77gm. m.pt. 36-38°C.) was obtained by crystallisation of the oil from light petroleum at 0°C. Recrystallisation and working up of the mother liquors gave the pure acid (68gm. m.pt. 38.5-39.5°C.) as shiny white plates and a further quantity (10gm.) of crude acid.

In view of the high proportion of santalbic acid present in the oil, santalbic acid was obtained directly from the seeds, without intermediate extraction. (This method saves time but the yield is not so high).

30gm. of seeds were crushed in a mortar and refluxed with alcoholic potash (6gm. KOH/6c.c. H_2O and 150c.c. alcohol) for one hour, the solution assuming a deep yellow colour. About 50c.c. of alcohol was distilled off and the remaining solution filtered. The residue remaining on the filter paper was well washed with water. It was found that the filtrate became very dark red in colour (i.e. on addition of water). Sulphuric acid (25c.c.: 25%) was then added and the solution extracted with ether (3 x 150c.c.); at this stage an emulsion was formed which did not entirely settle on standing. The clear ethereal solution was decanted off and the remainder, which was in suspension, was shaken with anhydrous magnesium sulphate. This procedure seemed to be successful in breaking the emulsion and most of the yellow ether extract was finally obtained by decantation. On drying this ethereal solution, removal of the solvent gave an orange yellow oil (10.6gm.) which later solidified. Dissolved in light petroleum (50c.c.) and allowed to crystallise at 0°C. the oil gave a pale yellow solid. By redissolving this solid in light petroleum (50c.c.) and purifying by refluxing with animal charcoal,

filtration and cooling slowly to 0°C. gave the characteristic white plates of santalbic acid (5.8gm., m.pt. 37-39°C).

The p-bromophenacyl ester, prepared by standard methods, was obtained as white crystals m.pt. 53-54°C. (Found: C, 65.6; H, 7.6; Br 17.1%. Calc. for $C_{24}H_{35}O_3Br$: C, 65.7; H, 7.4; Br, 16.8%). [cf. m.pt. of ester from ximenynic acid 53.5-54°C (Hatt and Szumer 1954)].

Determination of Unsaponifiable Material

The unsaponifiable material in the oil was determined by the standard method 'Sub-Comm. on Detn. of Unsap. Matter in Oils and Fats 1933). This material was only sparingly soluble in ether and similar solvents and was present to the extent of 6.9% in the oil (From ultraviolet data the unsaponifiable matter in the oil is 7%).

Ultraviolet Absorption Spectra

In the case of the oil, spectroscopic hexane was used as the solvent, and as the pure acid has $E^{1\%}_{1cm}$ at 229mμ of 596, the content of santalbic acid could be determined by measuring the absorption at 229mμ (the maximum). In this way, the santalbic acid content of the mixed acids excluding unsaponifiable is 95% ($E^{1\%}_{1cm}$ 570) and of the oil itself (as glyceride) 88% ($E^{1\%}_{1cm}$ 497).

Hydrogenation and Oxidation of Santalbic Acid

As these reactions had been adequately carried out in a series of preliminary experiments they were not repeated in this work. (see Gunstone and McGee 1954; Gunstone and Russell 1955).

Preparation of 11:12 Dihydroxystearolic Acid (II)

To santalbic acid (22gm.) dissolved in formic acid (98-100%; 240c.c.) by stirring at 28°C., 12c.c. of 100 volume hydrogen peroxide was added and the solution stirred vigorously for about 2½ hours at 40°C. Some of the formic acid (150c.c.) was then removed under reduced pressure at 40°C., and the residue after addition of plenty of water extracted with ether (3 x 200c.c.). Removal of the solvent left the hydroxyformoxy esters which were hydrolysed by refluxing with 3N aqueous sodium hydroxide (150c.c.). The soaps were then poured into 250c.c. of hot 3N hydrochloric acid and the resulting oil, which separated out, was extracted with ether (3 x 150c.c.) and the solution dried with anhydrous magnesium sulphate. The solvent was removed giving a dark red oil which was crystallised from ether - light petroleum (1:1; 200c.c.) to give the crude dihydroxystearolic acid (8.4gm.) as a yellow powder. This material was then dissolved in ethyl acetate and refluxed with animal charcoal for 5 minutes. After filtration, the resulting colourless solution was allowed to stand at 0°C., yielding a white solid which on further recrystallisation from ethyl acetate gave 4.8gm. of a white powder m.pt. 87-88°. A sample of this was recrystallised from alcohol to give a microcrystalline white powder m.pt. 88-89°C. (Grigor et al., 1955 quote 89-90°C.). Found: C, 69.5; H, 10.2%. Calc. for $C_{18}H_{32}O_4$: C, 69.2; H, 10.3%).

Hydrogenation of 11:12 Dihydroxystearolic Acid

Dihydroxystearolic acid (2gm.) was dissolved in alcohol (50c.c.) and palladium charcoal (300mg.) added, the resulting suspension being shaken with hydrogen until no more gas was absorbed (300c.c. at 20°C.; theoretical 285c.c. at N.T.P.). As the dihydroxystearic acid crystallised out during the hydrogenation, the solution was warmed and then filtered free of catalyst. The filtrate was then allowed to stand overnight at 0°C. Filtration gave a white powder (1.9gm.) which on recrystallisation from alcohol yielded 11:12 dihydroxystearic acid (III) as a white microcrystalline powder m.pt. 126-126.5°C. (Found: C, 68.3; H, 11.1%. Calc. for $C_{18}H_{36}O_4$ C, 68.4; H, 11.4%). A later preparation gave a sample m.pt. 127-128°C. A mixed m.pt. with erythro 9:10 dihydroxystearic acid (m.pt. 129-131°C.) gave a depression.

Oxidation of 11:12 Dihydroxystearic Acid

Dihydroxystearic acid (0.6gm.) was dissolved in glacial acetic acid (25c.c.) and excess of powdered potassium permanganate added (1.5gm.) in small portions at room temperature over a period of ½ hour, with gentle swirling of the flask. The temperature of the flask was slowly raised to 40°C. and kept about that temperature for 2 hours, with gentle shaking. Some of the acetic acid was then removed under reduced pressure, the solution decolorised with sulphur dioxide, dilute hydrochloric acid added and the solution steam distilled.

The residual solution from this steam distillation deposited a white solid when cold which, on recrystallisation from water gave white crystalline plates of 1:11 undecanedioic acid (0.3gm.; m.pt. 108.5-109.5°C). This sample had a mixed m.pt of 108.5-110°C. with the crystals obtained from the oxidation of 10-formyldecanoic acid (see page 173).
The steam volatile fraction on extraction with chloroform (3 x 50c.c.) yielded on removal of the solvent, a yellow oil (0.15gm.). The p-bromophenacyl ester of this acid was prepared as white crystals (from ethanol) m.pt. 68-70°C. A mixed m.pt. with the ester from an authentic sample of heptanoic acid (m.pt. 68-69°C.) gave no depression.

Periodate Oxidation of 11:12 Dihydroxystearic Acid

A solution of potassium periodate (1.5gm.) in N sulphuric acid (75c.c.) was added to the dihydroxystearic acid (2gm.) in ethanol (70c.c.) and the solution shaken at 40°C. for 15 minutes. Water (300c.c.) was then added and the solution extracted with ether (3 x 150c.c.). The solvent was removed and the product steam distilled for about 1½ hours, collecting about 250c.c. of distillate [chemical separation gave less satisfactory results].

The residual solution on dilution with water (150c.c.) was then extracted with ether (3 x 100c.c.). Drying with anhydrous magnesium sulphate and removal of the solvent gave 0.8gm. of a yellowish oil which solidified on cooling.

This was dissolved in light petroleum (50c.c.) and crystallised at 0°C. giving a white powder m.pt. 42.5-44°C. Further recrystallisation from light petroleum [b.pt. 60-80°C.: 30c.c.] gave 10-formyldecanoic acid (IIIB) as a white powder m.pt. 47-48°C. [Found: C,65.7; H, 9.9%. Calc. for $C_{11}H_{20}O_3$: C, 66.0; H, 10.1%]. The 2:4 dinitrophenylhydrazone of ethyl 10-formyldecanoate was obtained as yellow needles m.pt. 59-60°C. by dissolving the acid (IIIB) in ethanol with a sulphuric acid solution of the hydrazine hydrochloride. [Found: C, 55.7; H, 6.7; N, 13.9%. Calc. for $C_{19}H_{28}O_6N_4$: C, 55.9; H, 6.9; N, 13.7%]. An attempt was made to prepare the 2:4 dinitrophenyl hydrazone of the acid in glacial acetic acid; after purification by chromatography on a silica gel column, a small quantity of an orange yellow powder m.pt. 89-90°C. was obtained.

The steam volatile material was extracted with ether (3 x 100c.c.) giving 0.7gm. of a reddish yellow fragrant oil. The 2:4 dinitrophenyl hydrazone of this compound was prepared in the usual manner to give orange yellow plates m.pt. 103-104°C [Found: C, 53.3; H, 5.9%. Calc. for $C_{13}H_{18}O_4N_4$: C, 53.1; H, 6.2%]. A mixed m.pt. with a sample of the 2:4 dinitrophenylhydrazone of authentic heptanal (m.pt. 105-105.5°C) gave m.pt. 104.5-105°C. [It was noted during the preparation

of the above derivatives that orange-yellow compounds could be obtained fairly readily from the aldehyde and from authentic heptanal but the m.pts. were a little lower than that expected (about 95°C.)].

Another portion of the aldehydo acid (III B) was submitted to oxidation by potassium permanganate:- 10-Formyldecanoic acid (300mg.) was suspended in dilute sulphuric acid (60 c.c. of 1.N) and an excess of powdered potassium permanganate added (0.8gm.). The temperature of the solution was gradually raised to 50°C. and the solution shaken at this temperature for 45 minutes. Decolorisation with sulphur dioxide followed by filtration gave about 0.2gm. of a pale brown material which was dissolved in alcohol (15c.c.) and refluxed with animal charcoal for 5 minutes. Filtration gave a colourless solution which deposited a white powder m.pt. 105-107°C. Further crystallisation from nitromethane gave 1:11 undecanedioic acid as microcrystalline white needles m.pt. 109-110°C. [Lit. 110°C.]. [Found: C, 60.9; H, 9.6%. Calc. for $C_{11}H_{20}O_4$: C, 61.1; H, 9.3%].

Periodate Oxidation of 11:12 Dihydroxystearolic Acid

2 gm. of the dihydroxystearolic acid was submitted to oxidation with potassium permanganate in sulphuric acid as outlined above for dihydroxystearic acid.

The aldehydo acid obtained from the residual solution of the steam distillation by extraction with light petroleum yielded a small quantity of a white crystalline material. This was further crystallised from light petroleum to give white crystalline plates of 10-formyldec-9-ynoic acid (IV) m.pt. 25.5-26.5°C. [Found: C, 67.3; H, 7.9% for $C_{11}H_{16}O_3$: C, 67.3; H, 8.2%]. It is recommended that this reaction be carried out as quickly as possible since the aldehydo acid seems to polymerise readily. Measurement of the U.V. absorption of this compound in rectified spirits showed a broad band with a maximum about 231mμ ($E^{1\%}_{1cm}$ 206), while measurement in n-hexane gave slightly different results (see page 158). (This latter measurement was made a few weeks later on a sample kept in a stoppered tube at 0°C.). A sample of the 2:4 dinitrophenylhydrazone of this compound was prepared in glacial acetic acid solution. Addition of water gave a bright yellow material which on recrystallisation from ethanol deposited a yellow powder (50gm.) m.pt. 82-85°C. This powder was purified by a chromatographic procedure:- the material was dissolved in benzene (2c.c.) and placed on top of a silica gel (100 mesh) column (20cm) which was then eluted with benzene. The first fifty c.c.s. of eluent dislodged a pale yellow band, and on changing the eluent gradually to 50% benzene-chloroform solution a deep yellow band was eluted, which on evaporation of the solvent and

crystallisation from ethanol gave 10mg. of a deep yellow powder - the 2:4 dinitrophenylhydrazone of 10-formyldec-9-ynoic acid (IV) m.pt. 105.5-106.5°C. [Found: C, 53.9; H, 5.3; N, 14.7%. Calc. for $C_{17}H_{20}O_6N_4$: C, 54.2; H, 5.4; N, 14.9%].

The steam volatile fraction on extraction with ether and removal of the solvent gave a yellow fragrant oil (0.5gm.). The 2:4 dinitrophenylhydrazone of this oil was prepared in the usual way giving orange yellow needles m.pt. 105-105.5°C. [lit. 106°C.]. [Found: C, 53.2; H, 6.0; N, 18.8%. calc. for $C_{13}H_{18}O_4N_4$; C, 53.1; H, 6.2; N, 19.0%]. The semi-carbazone of this fraction was also prepared and obtained as white crystals m.pt. 108-108.5°C. from ethanol. Mixed m.pt. with a sample prepared from authentic heptanal (m.pt. 107-108°C).gave 107.5-108.5°C.

Preparation of Lindlar's Catalyst

Calcium carbonate (50gm.: 'Analar') was mixed into distilled water (400c.c.) and 50c.c. of a solution of palladium chloride (4.15gm.: 5%) were added whilst stirring at room temperature for 5 minutes and then for 10 minutes at 80°C. The hot suspension was shaken with hydrogen until no more was absorbed. [4 hours at 20°C.: 1760c.c. H_2]. The solid was filtered off, well washed with distilled water (4 litres) and then suspended in 500 c.c. distilled water and vigorously stirred whilst adding a solution of lead acetate

(5gm. in 100c.c. water). Stirring was continued at room temperature for 10 minutes and for 40 minutes on a steam bath. The catalyst was then filtered, washed with distilled water (6 litres) and placed in a vacuum desiccator to dry at 40°C. for 8 hours. This produced 49gm. of a grey powder which was used as a poisoned catalyst in conjunction with quinoline.

Partial Hydrogenation of Santalbic Acid

Santalbic acid (2 gm.) was dissolved in ethanol (30c.c.) 200mg. of Lindlar's catalyst and 80mg. of quinoline added. This suspension was shaken with hydrogen in the usual way. There was a slow but steady uptake of hydrogen and after 200 c.c. of hydrogen had been absorbed at 20°C the reaction was stopped. [The uptake of hydrogen showed no signs of diminishing (cf. Crombie 1955b); theoretical value for hydrogen absorbed in the reduction of one triple bond to one double bond is 175 c.c. at 20°C.]. The suspension was filtered and the alcohol solution allowed to stand overnight at -20°C. A white solid crystallised out, but as this appeared to melt at about 0°C., the solvent was removed and the residue replaced in the refrigerator where it solidified. This solid on examination by ultra-violet spectroscopy showed a broad band with a maximum at 231mμ ($E^{1\%}_{1cm}$ 856) and a weak point of inflexion at 240mμ (see page 159). These properties are consistent with this solid being octadec-*cis*-9-*trans*-11-dienoic acid (when the hydrogenation was carried

out in light petroleum there appeared to be no uptake of hydrogen).

Isomerisation of Octadec-cis-9-trans-11-dienoic acid

The cis-trans acid (2gm.) was dissolved in 500c.c. light petroleum and 15c.c. of a solution of iodine ($N/_{100}$) in the same solvent added. The solution was then placed in bright sunlight for 3 hours (or irradiated with u.v. light for ½ hour), when the violet colour of the iodine disappeared. The residue, after removal of the solvent, was then crystallised from alcohol giving white crystalline plates (1gm.) m.pt. 48-50°C. Further recrystallisation from alcohol was carried out giving white plates m.pt. 53-54°C. of octadec-trans-9-trans-11 dienoic acid. (Mangold's acid). [Found: C, 76.9; H, 11.5%. calc. for $C_{18}H_{32}O_2$: C, 77.1; H, 11.5%]. The acid had a U.V. absorption curve with a maximum at 231mμ ($E^{1\%}_{1cm}$ 1198) and two points of inflexion at 227mμ and 239mμ (see pages160,161).

The maleic anhydride adduct of the acid was prepared:- 250mg. of acid and 80mg. of maleic anhydride were mixed and heated to 75°C. on a water bath. The mixture was kept at this temperature for 2½ hours. The oil produced was dissolved in petroleum ether (b.pt. 60-80°C.) and placed in the refrigerator. This produced a white crystalline material m.pt. 78-82°C. On redissolving in petroleum ether a part of the material appeared to be fairly insoluble and this

less soluble compound was filtered off giving white crystalline plates m.pt. 88-90°C. Further recrystallisation from petroleum ether gave white crystalline plates m.pt. 92.5-93.5°C. [Found: C, 69.8; H, 8.8%. Calc. for $C_{22}H_{34}O_5$: C, 69.8; H, 9.1%]. [literature: 88°C. (Boëseken and Hoevers 1930); 94.5°C. (Kaufmann and Baltes 1936) 78-96°C. (Schmid and Lehmann 1950)].

Partial Hydrogenation of 11:12 Dihydroxystearolic Acid

Dihydroxystearolic acid (1.0gm.) was dissolved in alcohol (30c.c.), 100mg. of Lindlar's catalyst and 40mg. of quinoline added. This suspension was then shaken with hydrogen until 90c.c. of hydrogen was absorbed (theoretical value 78 c.c. at 21°C.). On filtration and removal of the solvent, the residue was dissolved in light petroleum. Repeated crystallisation from this solvent and finally from nitromethane gave erythro 11:12 dihydroxyoctadec-cis-9-enoic acid (V) (dihydroxyoleic acid) as microcrystalline white needles m.pt. 68-68.5°C. [Found: C, 68.6; H, 10.8%. Calc. for $C_{18}H_{34}O_4$: C, 68.8; H, 10.9%: Microhydrogenation shows 1.1 double bonds].

Lithium Aluminium Hydride Reduction of 11:12 Dihydroxystearolic Acid

The dihydroxystearolic acid (1gm.) was suspended in dry ether (20c.c.) and slowly added to a suspension of lithium aluminium hydride (0.95gm.: 7.5gm. mol.) in 40c.c. of dry ether, whilst stirring. The suspension was refluxed on a water bath for 2¾ hours and then allowed to stand overnight. Ethyl acetate (10c.c.) and cold 3N hydrochloric acid (20c.c.) were

added to the suspension which was then extracted with ether (3 x 100c.c.). This ether solution was washed with 10% potassium hydroxide solution (3 x 20c.c.) to remove any unchanged acid, and then with water until free of alkali (tested with phenolphthalein). On drying with anhydrous magnesium sulphate and removing the solvent a white solid (1 gm.) m.pt. 73-75.5°C. was obtained. Further crystallisation from alcohol gave 1:11:12 trihydroxyoctadec-9-yne (VI) m.pt. 79-80°C as a white powder. (Found: C, 72.1; H, 11.5%. Calc. for $C_{18}H_{34}O_3$: C, 72.4; H, 11.5% . Microhydrogenation indicated 0.9 triple bond or 1.82 double bonds).

Increasing the quantity of lithium aluminium hydride [10gm. mol ⟶ 16gm mol.] and varying the time of reflux (1 hour: 11 Hours) did not change the nature of the product.

Preparation of 1:11:12 Trihydroxyoctadec-9-yne from Santalbic Acid

Santalbic acid (1.6gm.) was dissolved in dry ether (25c.c.) and slowly added to a suspension of lithium aluminium hydride (0.3gm.: 5gm. mol) in dry ether (30c.c.) and refluxed for 1 hour whilst stirring. The suspension was allowed to stand overnight and worked up the usual way (as for VI) giving a yellow oil (0.9gm.), a sample (0.3gm.) of which on crystallisation from petroleum ether (b.pt. 80-100°C.) gave ximenynyl alcohol (VIII) as a white solid m.pt. 23-25°C. (lit. 30-31°C.). The α-naphthyl urethane derivative was prepared and gave white crystals from petroleum ether (b.pt. 60-80°C.) m.pt. 56-57°C.

(lit. 57-57.5°C.).

Ximenynyl alcohol (0.6gm.) was suspended in 5c.c. formic acid (98-100%) and 0.3c.c. of 10⁰ volume hydrogen peroxide added. The mixture was shaken at 40°C. for about 2 hours (assuming a reddish yellow colour) and a quantity of formic acid then removed under reduced pressure at 40°C. The residue was hydrolysed by refluxing on the steam bath with aqueous 3N sodium hydroxide solution (2c.c.) for 5 minutes. The resulting solution was diluted with water (50c.c.) and extracted with ether (3 x 30c.c.), the ethereal solution being dried with anhydrous magnesium sulphate. Removal of the solvent gave 0.25gm. of a yellow oil which solidified on placing in the refrigerator. Recrystallisation from ethyl acetate yielded a small quantity of a white powder m.pt. 76-77°C. A mixed m.pt. with compound VI obtained by lithium aluminium hydride reduction of dihydrostearolic acid (m.pt. 78-79°C.) gave m.pt. 77-79°C., thus confirming that VI is the -yne triol.

Preparation of 1:11:12 Trihydroxyoctadecane (VII)

a) Dihydroxystearic acid (III) (0.15gm.) was dissolved in dry ether (10c.c.) and slowly added to a suspension of lithium aluminium hydride (0.2gm.: 18gm. mol.) in dry ether (20c.c.) whilst stirring. The suspension was refluxed for 2 hours and then worked up as usual (as for VI) giving a white powder (0.1gm.) m.pt. 124-125°C. Further recrystallisation from

ethanol gave 1:11:12 trihydroxyoctadecane (VII) as a white powder m.pt. 126-126.5°C. [Found: C, 71.6; H, 12.5%. Calc. for $C_{18}H_{38}O_3$: C, 71.5; H, 12.7%].

b) 1:11:12 Trihydroxyoctadec-9-yne (VI) (0.1gm.) was dissolved in ethanol (20c.c.) and palladium charcoal (20 mg.) added. The suspension was shaken with hydrogen until no more hydrogen was absorbed. On filtration and evaporation of the solvent a white powder (VII) was obtained m.pt. 122-123.5°C.

c) 11:12 Dihydroxyoleic acid (V) (0.2gm.) was dissolved in dry ether (20c.c.) and added slowly to a suspension of lithium aluminium hydride (0.1 gm.: 4.6gm. mol.) in dry ether (20c.c.). The suspension was then refluxed for 3 hours and the trihydroxy compound extracted as in (VI) to yield 0.1gm. of an impure white solid. Crystallisation from petroleum ether (b.pt. 60-80°C.) gave a small quantity of a white powder m.pt. 51-52°C which was probably 1:11:12 trihydroxyoctadec-cis-9-ene (IX), since microhydrogenation in glacial acetic acid (H_2 uptake ≡ 0.94 double bond) gave a white powder m.pt. 122-124°C (VII).

Samples of VII obtained via a), b) and c) when mixed together did not show any depression of m.pts.

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